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STUDIES ON THE VITAMIN CONTENT OF TISSUES II

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The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

Sam Houston

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man. It is the only dictator that freemen acknowledge, and the only security which freemen desire.

Mirabeau B. Lamar.

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THE AUTHORS

INTRODUCTION—MICROBIOLOGICAL ASSAY METHODS

By

Roger J. Williams

In a former bulletin from this laboratory we made use of a series of microbiological tests for the study of the vitamin content of tissues. Each of the testing methods was described in detail and three papers in which the testing methods were involved were included.

The present bulletin is an extension of these studies in which essentially the same methods have been used. The method of preparing extracts through the use of enzymatic digestion has been greatly improved and yields which are more nearly quantitative are obtained. We cannot be sure even yet that the total amount of vitamins of a tissue are released by the method used. In the case of biotin, for example, acid hydrolysis releases more from some tissues than the enzyme treatment, but on the other hand enzyme release is more effective for certain other tissues.

The methods themselves are capable of improvement and we expect to investigate them more fully in the near future. Methods which are applicable to certain types of material may need modification before they can be used successfully with materials of an entirely different nature.

In the present bulletin we are not repeating the complete description of the different assay methods, but are giving the essential information regarding each, so that anyone accustomed to carrying out tests of this kind can apply the methods.

RIBOFLAVIN ASSAY

Organism.—*Lactobacillus casei* ε.

Basal medium.—(The concentration of this medium is twice the desired final concentration.)

Sodium-hydroxide treated peptone (plus sodium acetate)	10 gm.
Cystine hydrochloride	200 mg.
Yeast Supplement	2 gm.
Glucose	20 gm.
Inorganic Salts—Solutions A and B	10 ml. each
Distilled water to one liter.	

Standard dosages.—0.05 to 0.5 microgram riboflavin per 10 ml. final medium.

Inoculation.—1 drop of a suspension.

Time of incubation.—24–72 hours at 37° C.

Measurement of response.—Turbidity, 24 hours; Titration, 48–72 hours.

Alternative method.—1/50 scale, grown in spot-plate depressions. Microtitration (drop scale).

Reference.—Snell, E. E., and Strong, F. M., *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939). Snell, E. E., and Strong, F. M., *The University of Texas Publication*, No. 4137, 11 (1941).

Additional Reference.—Landy, M. and Dicken, D. M., *J. Lab. Clin. Med.*, **27**, 1086 (1942).

PANTOTHENIC ACID ASSAY

Organism.—*Lactobacillus casei* ϵ.

Basal medium.—(The concentration of this medium is twice the desired final concentration.)

Alkali-treated peptone (plus sodium acetate)	10 gm.
Glucose	20 gm.
Alkali-treated yeast extract	2 gm.
Acid-hydrolyzed casein	4 gm.
Cystine hydrochloride	200 mg.
Riboflavin	200 γ
Inorganic Salts—Solutions A and B	10 ml. each

Distilled water to one liter.

Standard dosages.—0.01 to 0.20 microgram calcium pantothenate per 10 ml. final medium.

Inoculation.—1 drop of a suspension.

Time of incubation.—24–72 hours at 37° C.

Measurement of response.—Turbidity, 24 hours; Titration, 72 hours.

Alternative method.—1/50 scale, grown in spot-plate depressions. Microtitration (drop scale).

Reference.—Pennington, D., Snell, E. E. and Williams, R. J., *J. Biol. Chem.*, **135**, 213 (1940).

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Landy, M. and Dicken, D. M., *J. Lab. Clin. Med.*, **27**, 1086 (1942).

BIOTIN ASSAY*

Organism.—*Saccharomyces cerevisiae* F. B., isolated from Fleischmann's yeast (pound goods).

Basal medium.—

Sucrose	20 gm.
$(NH_4)_2SO_4$	3 gm.
KH_2PO_4	2 gm.
l-Aspartic acid	0.1 gm.
$CaCl_2$	0.25 gm.
$MgSO_4.7H_2O$	0.25 gm.
H_3BO_3, $ZnSO_4$, $MnCl_2$, $TlCl_3$	1 mg. each
$FeCl_3$	0.5 mg.
$CuSO_4.5H_2O$	0.1 mg.
KI	0.1 mg.
Inositol	5 mg.
β-Alanine	0.5 mg.
Thiamin hydrochloride	20 γ
Pyridoxin hydrochloride	20 γ

Distilled water to one liter.

Standard dosages.—10–100 micromicrograms (10^{-12} gm.) of biotin methyl ester per 6 ml. of final medium. Standard and samples are diluted to a volume of 1 ml.

---

*Biotin values determined by this test vary somewhat with the pH of the basal medium, especially when relatively poor biotin sources are being tested. This effect is not yet elucidated and will be subjected to further study.

Inoculation.—5 ml. of a suspension in medium containing 0.01 mg. moist yeast.

Time of incubation.—16 hours at 30° C.

Measurement of response.—Turbidity.

Reference.—Snell, E. E., Eakin, R. E. and Williams, R. J., *J. Am. Chem. Soc.*, **62**, 175 (1940).

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Landy, M. and Dicken, D. M., *J. Lab. Clin. Med.*, **27**, 1086 (1942).

NICOTINIC ACID ASSAY

Organism.—*Lactobacillus arabinosus* 17–5.

Basal medium.—(The concentration of this medium is twice the desired final concentration.)

Vitamin-free hydrolyzed casein	10	gm.
Tryptophane	200	mg.
Cystine	200	mg.
Glucose	20	gm.
Sodium Acetate	12	gm.
Adenine sulfate	20	mg.
Guanine hydrochloride	20	mg.
Uracil	20	mg.
Thiamin hydrochloride	200	γ
Pyridoxin hydrochloride	200	γ
Calcium pantothenate	200	γ
Biotin	0.8	γ
Riboflavin	400	γ
Inorganic Salts—Solutions A and B	10	ml. each

Distilled water to one liter.

Standard dosage.—0.05–0.50 microgram nicotinic acid per 10 ml. of final medium.

Inoculation.—1 drop of a suspension.

Time of incubation.—24–72 hours at 30° C.

Measurements of response.—Turbidity at 24 hours; Titration at 72 hours.

Alternative method.—1/50 scale grown in spot-plate depressions. Microtitration (drop scale).

Reference.—Snell, E. E. and Wright, L. D., *J. Biol. Chem.*, **139**, 675 (1941).

Snell, E. E., and Wright, L. D., *The University of Texas Publication*, No. 4137, 22 (1941).

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Landy, M. and Dicken, D. M., *J. Lab. Clin. Med.*, **27**, 1086 (1942).

PYRIDOXIN ASSAY*

Organism.—*Saccharomyces cerevisiae.* "Gebrüder Mayer" (G.M.) strain.

Basal medium.—

*According to our experimental findings this test is specific for the well-recognized substance (synthetic) known as pyridoxin. The tests for "vitamin B_6" by animal assay may be seriously influenced by the existence of "pseudopyridoxin." (Snell, E. E., Guirard, B. M., and Williams, R. J., *J. Biol. Chem.* **143**, 519 (1942).

Sucrose	20	gm.
KH_2PO_4	2	gm.
$(NH_4)_2SO_4$	3	gm.
l-Asparagine	0.75	gm.
$CaCl_2$	0.25	gm.
$MgSO_4.7H_2O$	0.25	gm.
H_3BO_3, $ZnSO_4$, $MnCl_2$, $TlCl_3$	1	mg. each
$FeCl_3$	0.5	mg.
$CuSO_4.5H_2O$	0.1	mg.
KI	0.1	mg.
Vitamin-free hydrolyzed casein	0.2	gm.
Biotin	0.2	γ
Calcium pantothenate	300	γ
Folic acid	0.5	γ
Inositol	5	mg.
Thiamin hydrochloride	20	γ

Distilled water to one liter.

Just before use, 2 ml. of each of the following supplements are added per 100 ml. basal medium:

Pyridoxin-Free Liver Extract—5.0 gm. of liver paste (Wilson Laboratories 1–20) is mixed with 195 ml. of water and autoclaved 15 minutes at 15 lbs. pressure, cooled to room temperature, and adjusted to pH 1.0 with HCl. Ten gm. of fuller's earth (Braun Corp.) is added and the mixture is shaken intermittently several times a day for four days. The supernatant liquid is then decanted, adjusted to pH. 4.5–5.0, and pipetted into test tubes (5 ml. portions) which are plugged and autoclaved 15 minutes at 15 lbs. Three days later the tubes are again autoclaved as before.

Pyridoxin-Free Yeast Extract—5.0 gm. of Difco Bacto yeast extract is treated in the same way as the liver paste above.

Standard dosage.—0.2–0.4 millimicrograms per 2 ml. of medium. Volumes up to 0.1 ml. for each 2 ml. of basal medium may be used.

Inoculation.—2 ml. of a suspension in basal medium containing 0.001 mg. of moist yeast.

Time of incubation.—15 hours at 30° C.

Measurement of response.—Turbidity.

Reference.—Williams, R. J., Eakin, R. E., and McMahan, J. R., *The University of Texas Publication*, No. 4137, 24 (1941).

INOSITOL ASSAY

Organism.—*Saccharomyces cerevisiae*, "Gebrüder Mayer" (G.M.) strain.

Basal medium.—

Sucrose	20	gm.
KH_2PO_4	2	gm.
$(NH_4)_2SO_4$	3	gm.
l-Asparagine	0.75	gm.
$CaCl_2$	0.25	gm.
$MgSO_4.7H_2O$	0.25	gm.
H_3BO_3, $ZnSO_4$, $MnCl_2$, $TlCl_3$	1	mg. each
$FeCl_3$	0.5	mg.
$CuSO_4.5H_2O$	0.1	mg.
KI	0.1	mg.
Vitamin-free hydrolyzed casein	0.2	gm.
Biotin	0.2	γ
Calcium pantothenate	300	γ
Folic acid	0.5	γ
Thiamin hydrochloride	20	γ
Pyridoxine hydrochloride	20	γ

Distilled water to one liter.

Just before use, add 1 ml. of the following supplement per 100 ml. of basal medium:

Calf's Liver Supplement.—This supplement is made up by autolyzing calf's liver and is adjusted to a final concentration equivalent to 75 mg. of tissue per ml. The extract is put in plugged tubes, autoclaved 15 minutes at 15 lbs., and stored in a cool place. This extract should be assayed, and if it contains over 500 micrograms of inositol per gram of liver, it should not be used.

Standard dosage.—0.1–0.8 micrograms of inositol per 2 ml. of basal medium. Volumes up to 0.1 ml. per ml. of basal medium may be used.

Inoculation.—2 ml. of a suspension in basal medium containing 0.003 mg. moist yeast.

Time of incubation.—15 hours at 30° C.

Measurement of response.—Turbidity.

Reference.—Williams, R. J., Stout, A. K., Mitchell, H. K., and McMahan, J. R., *The University of Texas Publication*, No. 4137, 27 (1941).

Additional References.—Woolley, D. W., *J. Biol. Chem.*, **140**, 453 (1941).

THIAMIN ASSAY*

Organism.—*Saccharomyces cerevisiae*, "Old Process" (O.P.) strain.

Basal medium.—

Sucrose	20	gm.
KH_2PO_4	2	gm.
$(NH_4)_2SO_4$	3	gm.
l-Asparagine	0.75	gm.
$CaCl_2$	0.25	gm.
$MgSO_4.7H_2O$	0.25	gm.
H_3BO_3, $ZnSO_4$, $MnCl_2$, $TlCl_3$	1	mg.
$FeCl_3$	0.5	gm.
$CuSO_4.5H_2O$	1	mg.
KI	0.1	mg.
Inositol	5	mg.
Biotin	0.2	γ
Calcium pantothenate	300	γ
Pyridoxin hydrochloride	20	γ
Folic acid	0.5	γ
Vitamin-free hydrolyzed casein	0.4	gm.†
Distilled water to one liter.		

Standard dosage.—0.0002–0.002 micrograms of thiamin hydrochloride per 2 ml. basal medium. Volumes up to 0.1 ml. per 2 ml. of basal medium may be used.

Just before sterilizing the standard and sample tubes, add 0.4 ml. of the following buffered supplement to each tube:

Buffer Solution Containing Liver and Yeast Extracts which Have Been Freed from Thiamin—	
Thiamin-free liver extract (25 mg./ml.)	5 ml.
Thiamin-free yeast extract (25 mg./ml.)	5 ml.
Buffer to make	50 ml.

*This assay is also affected by the hydrolytic products of thiamin.

†This amount of hydrolyzed casein is double the amount previously recommended for this assay.

Thiamin-Free Liver Yeast Extracts.—5 gm. of liver paste (Wilson Laboratories (1–20) are added to 195 ml. of water, autoclaved for 15 minutes and cooled to room temperature. The pH is adjusted to approximately 3.0. Ten gm. of fuller's earth are added, the mixture is shaken for 30 minutes, filtered, and the pH is adjusted to approximately 1.0. After autoclaving again for 15 minutes, and cooling, a second 10 gram portion of fuller's earth is added and the mixture is shaken for at least two days. At the end of this time it is filtered, the pH is adjusted to 4.5–4.8 and filtered again. The filtrate is divided into 5 ml. portions, placed in plugged test tubes, autoclaved 15 minutes and stored in the refrigerator until ready for use.

The preparation of this supplement is the most troublesome part of this assay. There is considerable difference among various fuller's earths, and some are not at all effective. A preparation obtained from Braun Corp. has been used in this laboratory.

Inoculation.—2 ml. of a suspension in basal medium containing 0.0016 mg. of moist yeast.

Time of incubation.—15 hours at 30° C.

Measurement of response.—Turbidity.

Reference.—Williams, R. J., McMahan, J. R., and Eakin, R. E., *The University of Texas Publication*, No. 4137, 31 (1941).

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FOLIC ACID ASSAY

Organism.—*Streptococcus lactis* R.

Basal medium.—(The concentration of this medium is twice the desired final concentration.)

Vitamin-free hydrolyzed casein (Charcoal treated Darco G–60)	10	gm.
Sodium Acetate	12	gm.
Glucose	20	gm.
Tryptophane	100	mg.
Adenine sulfate	20	mg.
Cystine hydrochloride	200	mg.
Guanine hydrochloride	20	mg.
Xanthine	20	mg.
Uracil	20	mg.
Thiamin hydrochloride	200	γ
Pyridoxin hydrochloride	200	γ
Calcium pantothenate	200	γ
Riboflavin	400	γ
Nicotinic acid	200	γ
Biotin	0.4	γ
Inorganic Salts—Solutions A and B	5	ml. each

Distilled water to one liter.

Standard dosage.—0.0005–0.005 micrograms per 10 ml. of final medium of material having a "potency" of 40,000.

Inoculation.—1 drop of a suspension.

Time of incubation.—16 hours at 30° C.
Measurement of response.—Turbidity.

Reference.—Mitchell, H. K., and Snell, E. E., *The University of Texas Publication*, No. 4137, 36 (1941).
Additional Reference.—Landy, M. and Dicken, D. M., *J. Lab. Clin. Med.*, **27**, 1086 (1942).

STOCK SOLUTIONS

Inorganic Salts (for use in the Riboflavin, Pantothenic Acid and Nicotinic Acid assays).

Solution A—

K_2HPO_4	25	gm.
KH_2PO_4	25	gm.
H_2O to make	250	ml.

Solution B—

$MgSO_4.7H_2O$	10	gm.
NaCl	0.5	gm.
$FeSO_4.7H_2O$	0.5	gm.
$MnSO_4.4H_2O$	0.5	gm.
H_2O to make	250	ml.

Salts precipitate from solution B when it stands in air. It need be renewed only when a uniform suspension can no longer be obtained by shaking.

Alkali-treated Peptone (for use in Riboflavin, Pantothenic Acid and Nicotinic Acid assays).—40 gm. of Bacto-Peptone (Difco) in 250 ml. of water is treated with 20 gm. of sodium hydroxide dissolved in 250 ml. of water. The mixture (1 N in NaOH) is allowed to stand at 37° for 24 hours. The sodium hydroxide is neutralized with glacial acetic acid (27.9 ml.), 7 gm. of anhydrous sodium acetate is added and the mixture is diluted to 800 ml. The solution may be preserved under toluene.

ENZYMATIC LIBERATION OF B VITAMINS FROM PLANT AND ANIMAL TISSUES

By

Vernon H. Cheldelin, Margaret A. Eppright,
Esmond E. Snell and Beverly M. Guirard

The rapid accumulation of knowledge during recent years concerning the various B vitamins has stimulated research to determine the extent to which these substances occur in nature. Assay methods have been devised for a number of these, and in certain cases (notably riboflavin and thiamin) sufficient information has been collected to make possible at least a partial standardization of assay procedures.

Quantitative data regarding the remaining members of the B complex are much less complete. In certain cases discrepancies exist between the results obtained by different assay methods, and in several the optimal conditions for extraction of the vitamins from the tissues have not been found. Drastic chemical treatments, such as acid or alkali hydrolysis, which have been employed for freeing certain vitamins are not generally applicable since some of them are destroyed by these agents. Hot water extraction has often been used but is known to effect only incomplete extraction in many cases.

Among the milder procedures, autolysis and digestion with added enzymes seem attractive methods for releasing vitamins from tissues. Autolysis is substantially effective in a number of cases, and in the absence of further general evidence Williams and coworkers (1) employed autolysis routinely to free the B vitamins from animal tissues. Digestion with added enzymes has been employed in some cases but such treatment is not general.

The present study deals with seven vitamins: nicotinic acid, riboflavin, pantothenic acid, thiamin, inositol, biotin, and folic acid. For reasons of convenience and speed in making determinations we have chosen to employ microbiological methods of assay throughout.

EFFECT OF TIME OF AUTOLYSIS ON YIELD OF VITAMINS

A comparison of the yields of B vitamins from tissues as affected by time of autolysis is shown in Table I. Autolysates were prepared according to the method of Wright *et al.* (2).

Examination of Table I reveals certain differences in the rates of release of the B vitamins from the four tissues studied.

The amounts of biotin and nicotinic acid which can be extracted by autolysis are seen to reach a maximum within a relatively short time. Inositol is freed fairly rapidly also. Pantothenic acid, thiamin and pyridoxin are freed rapidly from beef muscle and green peas, but more slowly from hog heart and beef brain.

With the four tissues studied, assay values obtained by autolysis generally reached a maximum within twenty-four hours.

TABLE I

Yield of B Vitamins from Tissues as Affected by Length of Time of Autolysis
(γ/gm. of Fresh Tissue)

Tissue	Time of Autolysis	Thiamin Yield	Thiamin Relative Yield Max. 100%*	Nicotinic Acid Yield	Nicotinic Acid Relative Yield Max. 100%	Pantothenic Acid Yield	Pantothenic Acid Relative Yield Max. 100%	Pyridoxin Yield	Pyridoxin Relative Yield Max. 100%	Biotin Yield	Biotin Relative Yield Max. 100%	Inositol Yield	Inositol Relative Yield Max. 100%
Hog heart	30 min.	0.33	5.2	55	64	2.0	15	-----	----	0.050	100	990	65
	12 hrs.	5.2	83	86	100	8.9	68	0.070	70	0.032	64	1190	79
	24 hrs.	6.3	100	85	99	13	100	0.10	100	0.036	72	1510	100
Beef muscle	15 min.	0.22	69	48	80	3.2	97	----	----	0.0068	76	98	71
	1 hr.	0.30	94	----	----	2.3	70	0.10	71	0.0084	93	111	80
	2 hrs.	0.32	100	----	----	----	----	0.13	93	0.0090	100	107	78
	4 hrs.	-----	-----	58	97	3.1	94	0.14	100	0.0084	93	99	72
	8 hrs.	0.27	85	----	----	3.3	100	0.13	93	0.0086	96	100	73
	24 hrs.	0.25	78	60	100	3.3	100	----	----	0.008	89	116	84
	48 hrs.	0.30	94	59	98	----	----	----	----	-----	-----	138	100
Beef brain	0†	0.60	30	38	127	8.4	60	<0.01	7	0.032	100	1500	88
	12 hrs.	1.7	85	33	110	14	100	0.060	43	0.021	66	1700	100
	24 hrs.	2.0	100	30	100	10	71	0.14	100	0.025	78	1700	100
Green peas	0†	3.0	88	16	84	----	----	0.48	98	0.062	100	1040	80
	6 hrs.	3.0	88	19	100	----	----	0.38	78	0.050	81	980	75
	24 hrs.	3.4	100	18	94	----	----	0.46	94	0.052	84	1240	95
	48 hrs.	-----	-----	----	----	----	----	0.49	100	0.052	84	1300	100

*"Relative yield" in this table represents the per cent of the maximum yield obtained for each tissue by autolysis.
†Steamed 30 minutes.

EFFECT OF ADDED ENZYMES

Preparation of Extracts

Samples of fresh tissues were ground several times in a meat chopper, mixed thoroughly and weighed into sterile tubes or flasks. Each portion was suspended in ten times its weight of 1.0% buffer solution, the pH of which was chosen to coincide with the supposed "optimal pH" of each enzyme. For digestions with pepsin, 0.1% HCl was used; acetate buffer was used in the pH range 4.0–5.0; phosphate buffer from 6.0 to 7.0, and $NaHCO_3$ at pH 8.0–8.5. A weighed amount of each enzyme equal to 2% of the weight of the tissue sample was added to each flask, a few drops of benzene were added, and the samples were allowed to digest under specified conditions (Tables III to XI).

After digestion the samples were heated in flowing steam for 30 minutes to inactivate the enzymes and remove the benzene. Each sample was filtered through a very thin cake of "Filter Cel" on a Hirsch filter and the residue was washed with a volume of water equal to about twice that of the filtrate. The combined filtrates and washings were diluted, usually to a concentration of 25 mg. per ml. based upon the weights of the fresh tissues. The extracts were placed in tubes or flasks stoppered with cotton plugs, steamed five to ten minutes and stored in a dry-ice refrigerator until used.

In order to prevent destruction of riboflavin, the samples were protected from light as much as possible. Alkaline mixtures after trypsin or pancreatin digestion were acidified with 0.1% acetic acid before steaming, to prevent destruction of thiamin.

Materials which were received in a homogeneous state, such as milk powder, cereals and flour were not ground or mixed prior to the preparation of extracts.

Vitamin Contents of Enzymes

In Table II are listed the various B-vitamin contents of the enzyme preparations used in this study. In most cases the correction to be applied to tissue values is of little significance, since the enzymes represent only

TABLE II

B Vitamin Content of Enzymes
(in γ/gm.)

	Nicotinic Acid	Riboflavin	Pantothenic Acid	Thiamin	Inositol	Folic* Acid	Biotin
Takadiastase	20	2.5	9	0.59	9420	2.0	0.106
Malt diastase	22	3.0	41	2.2	1950	0.73	0.176
Pancreatic amylase	15	11	8.9	0.33	3820	0.43	0.076
Papain	14	6.2	81	1.3	2120	1.6	0.20
Pepsin	17	4.6	29	2.8	2510	0.33	0.13
Trypsin	26	7.7	36	1.3	5420	1.0	0.20
Pancreatin	5.9	4.7	13	0.40	2910	0.78	0.13

*Folic acid values are in terms of micrograms per gram of material having a "potency" of 40,000. (See page 12.)

two per cent of the total weight of the solids present when added singly, and four per cent in cases where two enzymes are used in combination.

THIAMIN

Thiamin assays were performed by the method of Williams, McMahan and Eakin (3).

It may be seen from Table III that the highest yields of thiamin are obtained generally with takadiastase and the other carbohydrases used. This observation is in line with the statement by Conner and Straub (4) that takadiastase and other related enzymes with high phosphatase activity are capable of hydrolyzing cocarboxylase quantitatively into free thiamin and phosphate. It appears that the phosphatases present in the tissues are themselves capable of releasing substantial amounts of the free vitamin

TABLE III

Effect of Enzyme Treatment on Yields of Thiamin from Various Tissues*
(γ/gm. of fresh tissue)

Material	Treatment	pH	Thiamin Content	Relative Yield Max. 100%
Hog Heart	Autolyzed 24 hrs. at 37°	natural	5.8	100
	Digested 24 hrs. at 37° with			
	malt diastase	4.5	5.0	86
	pancreatic amylase	7.0	5.0	86
	papain	5.0	4.6	79
	pepsin	2.1	4.4	76
	trypsin	8.4	3.6	62
Beef Leg Muscle	Autolyzed 24 hrs. at 37°	natural	0.46	38
	Digested 24 hrs. at 37° with			
	takadiastase	3.0	0.74	67
	malt diastase	4.5	1.1	100
	pancreatic amylase	7.0	0.83	69
	pepsin	1.8	0.64	53
	trypsin	8.3	0.44	37
	pancreatin	8.3	0.33	28
Beef Brain	Autolyzed 24 hrs. at 37°	natural	2.0	80
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	2.0	80
	malt diastase	4.5	2.5	100
	pancreatic amylase	7.0	1.2	48
	papain	5.0	1.9	76
	pepsin	2.1	2.1	84
	trypsin	8.4	0.68	27
	pancreatin	8.4	1.2	48
Green Peas	Autolyzed 24 hrs. at 37°	natural	3.7	69
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	5.4	100
	malt diastase	4.5	3.0	56
	pancreatic amylase	7.0	3.3	61
	papain	5.0	3.6	67
	pepsin	2.1	3.6	67

*In interpreting yields of the vitamins in this and other tables, it should be remembered that the microbiological methods give assay values which are generally reproducible to ± 10%. The magnitude of the actual assay difference is thus sometimes exaggerated if only "relative yields" are considered.

except in the case of beef muscle. Cocarboxylase, as previously pointed out (3), is inactive in this test.

Hog heart (Table I), although relatively rich in total thiamin apparently contains the greater portion in combined form, since only 5 per cent is extracted during the first half hour of autolysis. Similarly, the free thiamin in brain represents only about one-third of the total vitamin present. Prolonged autolysis of beef muscle and green peas, on the other hand, fails to produce increased yields. Since these samples were purchased in nearby markets it is possible that the tissue enzymes may have hydrolyzed the complexes before the extracts were prepared; otherwise one must assume the presence of relatively larger proportions of free thiamin in these materials.

The unusually high value obtained with malt diastase on beef muscle suggests the possible presence of combined forms of thiamin other than cocarboxylase. However, from the work of Westenbrink and coworkers (5) and Schäffner and Krumey (6) it seems doubtful that such complexes would be other esters of phosphoric acid, since various ortho- and pyrophosphates have been split by single enzyme preparations.

RIBOFLAVIN

The effect of enzymes upon the yields of riboflavin from various materials may be noted from Table IV. Extracts were assayed by the method of Snell and Strong (7).

It is apparent that such common methods for extraction of riboflavin as autoclaving with water or dilute acid are not complete in all cases. In the tissues studied, maximum yields are obtained by digestion with takadiastase or papain, as well as by a combination of the two enzymes.

TABLE IV

Effect of Enzyme Treatment on Yields of Riboflavin from Various Substances (γ/gm. of Fresh Tissue)

Material	Treatment	pH	Riboflavin Content	Relative Yield Max. 100%
Hog Heart	Digested 24 hrs. at 37° with			
	pancreatic amylase	7.0	6.9	87
	papain	5.0	7.9	100
	Steamed 30 minutes	natural	7.8	99
Beef Brain	Digested 24 hrs. at 37° with			
	takadiastase	4.5	1.4	100
	pancreatic amylase	7.0	1.3	93
	pepsin	2.1	1.4	100
	trypsin	8.4	1.3	93
	pancreatin	8.4	1.4	100
Egg Albumin	Autoclaved 30 minutes	natural	0.25	8.7
	Autoclaved 30 minutes with			
	1N H_2SO_4		2.56	90
	Autolyzed 24 hrs. at 37°	natural	0.52	18
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	2.54	89
	malt diastase	4.5	1.88	66
	papain	5.0	2.86	100

TABLE IV—Continued

Effect of Enzyme Treatment on Yields of Riboflavin from Various Substances
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Riboflavin Content	Relative Yield Max. 100%
Spinach	Autoclaved 30 minutes	natural	1.86	81
	Autoclaved 30 minutes with 1N H_2SO_4	—	2.27	99
	Autolyzed 24 hrs. at 37°	natural	1.81	79
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	2.03	88
	malt diastase	4.5	2.23	97
	papain	5.0	2.30	100
Carrots	Autoclaved 30 minutes	natural	0.20	35
	Autoclaved 30 minutes with 1N H_2SO_4	—	0.37	64
	Autolyzed 24 hrs. at 37°	natural	0.41	71
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	0.58	100
	malt diastase	4.5	0.57	98
	papain	5.0	0.55	95
Green Peas	Autolyzed 24 hrs. at 37°	natural	0.75	50
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	1.5	100
	malt diastase	4.5	1.2	80
	pancreatic amylase	7.0	1.5	100
	papain	5.0	1.2	80
	pepsin	2.1	1.3	87
	trypsin	8.4	1.0	67
	pancreatin	8.4	0.40	27
Whole Wheat Bread	Autoclaved, centrifuged	natural	1.1	100
	Autoclaved, then digested 24 hrs. at 37° with takadiastase	4.7	0.96	87
	Autoclaved, then digested 12 hrs. with takadiastase, then 12 hrs. with papain at 45–47°	4.7	1.1	100
	Autolyzed 24 hrs. at 37°	natural	0.96	87
Patent Flour	Autolyzed 24 hrs. at 37°	natural	0.34	100
	Autoclaved, then digested 12 hrs. with takadiastase, then 12 hrs. with papain at 45–47°	4.7	0.28	88
				Per cent Recovery
Riboflavin	Autoclaved 30 minutes with 0.1N H_2SO_4	—	—	95
	Autoclaved 30 minutes with 1N H_2SO_4	—	—	101

Efficiency of extraction by any single procedure differs with materials of different origin. Thus sulfuric acid releases virtually all the riboflavin from egg albumin and spinach but is relatively ineffective in releasing it from carrots. Maximum yields are obtained from spinach and carrots with malt diastase, but this enzyme releases only fractional amounts of the total riboflavin present in egg albumin. The reasons for such differences are not yet clear.

NICOTINIC ACID

The effects of enzyme treatment upon yields of nicotinic acid from various materials are listed in Table V. All assays were made by the Snell and Wright microbiological method (8).

TABLE V

Effect of Enzyme Treatment on Yields of Nicotinic Acid from Various Tissues*
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Nicotinic Acid Content	Relative Yield Max. 100%
Hog Heart	Autolyzed 24 hrs. at 37°	natural	85	98
	Digested 24 hrs. at 37° with			
	malt diastase	4.5	77	89
	pancreatic amylase	7.0	77	89
	papain	5.0	82	94
	pepsin	2.1	77	89
	trypsin	8.4	87	100
	pancreatin	8.4	74	85
	Digested 3 hrs. with takadiastase, then 3 hrs. with papain at 45–47°	4.7	81	93
	Steamed 30 minutes with O.1N HOAc		62	71
	Autoclaved 30 minutes	natural	79	91
Beef Leg	Autolyzed 24 hrs. at 37°	natural	41	89
	Digested 24 hrs. at 37° with			
	takadiastase	3.0	44	96
	malt diastase	4.5	46	100
	pancreatic amylase	7.0	46	100
	pepsin	1.8	46	100
	trypsin	8.4	46	100
	pancreatin	8.4	44	96
	Digested 3 hrs. with takadiastase, then 5 hrs. with papain at 45–47°	4.7	42	91
	Autoclaved 30 minutes	natural	40	87
Beef Brain	Autolyzed 24 hrs. at 37°	natural	30	86
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	30	86
	malt diastase	4.5	29	83
	pancreatic amylase	7.0	35	100
	papain	5.0	35	100
	pepsin	2.1	34	97
	trypsin	8.4	30	86
	pancreatin	8.4	35	100
	Digested 3 hrs. with takadiastase, then 3 hrs. with papain at 45–47°	4.7	35	100
	Autoclaved 30 minutes	natural	29	83
Green Peas	Autolyzed 24 hrs. at 37°	natural	20	91
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	20	91
	malt diastase	4.5	20	91
	pancreatic amylase	7.0	22	100
	papain	5.0	20	91
	pepsin	2.1	22	100
	trypsin	8.4	16	73
	pancreatin	8.4	21	96
	Digested 3 hrs. with takadiastase, then 5 hrs. with papain at 45–47°	4.7	20	91
	Autoclaved 30 minutes	natural	20	91

*pH adjusted to approximately 5 before steaming after digestion with enzyme.

TABLE V—Continued

Effect of Enzyme Treatment on Yields of Nicotinic Acid from Various Tissues
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Nicotinic Acid Content	Relative Yield Max. 100%
Milk Powder	Autolyzed 24 hrs. at 37°	natural	7.6	100
	Digested 24 hrs. at 37° with takadiastase	4.5	7.5	99
	papain	5.0	7.1	93
	Autoclaved 30 minutes	natural	6.2	82
	Autoclaved, then digested 24 hrs. at 37° with takadiastase	4.5	7.2	95
	Autoclaved, then digested 24 hrs. with papain at 37°	5.0	7.2	95
Cornmeal	Autolyzed 24 hrs. at 37°	natural	5.6	92
	Digested with takadiastase and papain together for 24 hrs. at 37°	4.7	5.7	94
	Autoclaved 30 minutes	natural	5.9	97
	Autoclaved, then digested 12 hrs. with takadiastase, then 12 hrs. with papain at 37°	4.7	6.1	100
Whole Wheat Bread	Autoclaved, then digested with takadiastase 3 hrs., then papain 5 hrs. at 45–47°	4.7	36	95
	Autoclaved, then digested 12 hrs. with takadiastase, then 12 hrs. with papain at 37°	4.7	38	100
	Autolyzed 24 hrs. at 37°	natural	23	61
	Autoclaved 30 minutes	natural	24	62
	Digested 24 hrs. at 37° with takadiastase	4.5	37	95
	clarase	4.5	39	100
	papain	5.0	37	95
	pepsin	1.5	35	90
	trypsin	8.5	37	95
White Flour	Autolyzed 24 hrs. at 37°	natural	4.3	54
	Autoclaved, then digested 3 hrs. with takadiastase, then 5 hrs. with papain at 45–47°	4.7	7.9	100
Brewers' Yeast, Dry	Autolyzed 24 hrs. at 37°	natural	490	100
	Autoclaved 30 minutes	natural	490	100
	Autoclaved, then digested 3 hrs. with takadiastase, then 5 hrs. with papain at 45–47°	4.7	490	100

Maximum amounts of nicotinic acid may be extracted from tissues by takadiastase or papain, either separately or in combination. The increases due to enzyme treatment are greatest for cereals; few if any significant increases occur with milk powder and meats, and it would appear to be satisfactory to omit the use of enzymes for these materials, relying upon autoclaving or autolysis to extract the vitamin.

Extraction with Acid and Alkali

It has been found (8, 9, 10, 11) that although animal tissues show essentially the same apparent nicotinic acid content regardless of method of extraction used, acid and alkaline extraction of cereals give higher results

than those obtained by autoclaving (8, 9, 10, 11) or by the use of enzymes (11). These higher values appear due to the presence in cereals of a nicotinic acid precursor which is very readily hydrolyzed to the free vitamin by alkali, less readily by acid, and only slowly by hot water (10,11). Chemical assay methods for nicotinic acid involve hydrolysis of such samples before colorimetric determination; values given by such methods check those obtained by the microbiological method after hydrolysis, but are higher than those secured by the microbiological method before hydrolysis. In preliminary experiments with cowpeas, on the other hand, digestion with a combination of takadiastase and papain has been found to give values as high or higher than those obtained with acid or alkali.

In view of these observations, it is not certain which method of extraction gives the correct values for nicotinic acid in a variety of foodstuffs.

Meanwhile we have chosen to employ enzyme digestion throughout because (a) the values obtained for most materials are the same by all of the common extraction methods and (b) in cases where differences exist, a number of different enzymes often give approximately the same value under varying conditions.

PANTOTHENIC ACID

Due to the instability of this vitamin toward treatment with acid or alkali, the comparative values in Table VI include only those obtained by autoclaving, autolysis or enzyme digestion. Assays of extracts were made by the method of Pennington, Snell and Williams (12).

TABLE VI

Effect of Enzyme Treatment on Yields of Pantothenic Acid from Various Materials (γ/gm. of Fresh Tissue)

Material	Treatment	pH	Pantothenic Acid Content	Relative Yield Max. 100%
Beef Brain	Autoclaved 30 minutes	natural	11	58
	Antolyzed 24 hrs. at 37°	natural	17	90
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	18	95
	malt diastase	4.5	13	68
	pancreatic amylase	7.0	16	84
	papain	5.0	18	95
	pepsin	2.1	13	68
	trypsin	8.4	10	53
	pancreatin	8.4	19	100
	Digested 3 hrs. with takadiastase, 5 hrs. with papain at 45–47°	4.7	18	95
Hog Heart	Autoclaved 30 minutes	natural	19	87
	Autolyzed 24 hrs. at 37°	natural	21	96
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	22	100
	malt diastase	4.5	19	87
	pancreatic amylase	7.0	19	87
	papain	5.0	17	77
	pepsin	2.1	14	64
	trypsin	8.4	12	55
	pancreatin	8.4	14	64
	Digested 3 hrs. with takadiastase, 5 hrs. with papain at 45–47°	4.7	22	100

TABLE VI—Continued

Effect of Enzyme Treatment on Yields of Pantothenic Acid from Various Materials
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Pantothenic Acid Content	Relative Yield Max. 100%
Beef Liver	Autolyzed 24 hrs. at 37°	natural	80	100
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	80	100
	malt diastase	4.5	79	99
	papain	5.0	78	98
Hog Kidney	Autolyzed 24 hrs. at 37°	natural	16	44
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	36	100
	malt diastase	4.5	15	42
	papain	5.0	16	44
Hog Leg Muscle	Autoclaved 30 minutes	natural	10	71
	Autolyzed 24 hrs. at 37°	natural	13	93
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	14	100
	malt diastase	4.5	11	78
	papain	5.0	12	86
Egg Albumin	Autoclaved 30 minutes	natural	6.1	100
	Autolyzed 24 hrs. at 37°	natural	4.8	79
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	6.1	100
	malt diastase	4.5	3.2	53
	papain	5.0	6.0	99
Spinach	Autoclaved 30 minutes	natural	1.9	95
	Autolyzed 24 hrs. at 37°	natural	1.7	85
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	2.0	100
	malt diastase	4.5	1.2	60
	papain	5.0	1.3	65
Carrots	Autolyzed 24 hrs. at 37°	natural	1.9	66
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	2.6	90
	malt diastase	4.5	2.0	69
	papain	5.0	2.9	100
	takadiastase and papain simultaneously	4.5	2.9	100
Green Peas	Autolyzed 24 hrs. at 37°	natural	2.7	87
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	3.0	97
	papain	5.0	3.1	100
	takadiastase and papain simultaneously	4.5	3.1	100
Whole Milk Powder	Autoclaved 30 minutes	natural	16	64
	Autolyzed 24 hrs. at 37°	natural	13	52
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	24	96
	papain	5.0	25	100
	Autoclaved, then digested 24 hrs. at 37° with			
	takadiastase	4.5	24	96
	papain	5.0	24	96

TABLE VI—Continued

Effect of Enzyme Treatment on Yields of Pantothenic Acid from Various Materials (γ/gm. of Fresh Tissue)

Material	Treatment	pH	Pantothenic Acid Content	Relative Yield Max. 100%
"Average American Diet"* — Batch No. 31	Autoclaved 30 minutes	natural	5.4	100
	Autoclaved, then digested 12 hrs. with takadiastase, 12 hrs. with papain at 37°	4.7	5.1	94
Batch No. 35	Autoclaved 30 minutes	natural	6.2	61
	Autoclaved, then digested 12 hrs. with takadiastase, 12 hrs. with papain at 37°	4.7	9.0	89
	Digested 24 hrs. with takadiastase and papain simultaneously at 37°	4.7	10.1	100
Whole Wheat Flour	Autoclaved 30 minutes	natural	9.6	100
	Autoclaved, then digested 3 hrs. with takadiastase, 5 hrs. with papain at 45–47°	4.7	9.3	97
	Digested 3 hrs. with takadiastase, then 5 hrs. with papain at 45–47°	4.7	9.0	94
Whole Wheat Bread	Autoclaved 30 minutes	natural	7.2	95
	Autoclaved, then digested 12 hrs. with takadiastase, 12 hrs. with papain at 37°	4.7	7.6	100
	Autolyzed 24 hrs. at 37°	natural	6.8	90
	Digested 24 hrs. at 37° with takadiastase and papain simultaneously	4.7	7.4	98
Cornmeal (Yellow)	Autoclaved 30 minutes	natural	2.9	100
	Autoclaved, then digested 12 hrs. with takadiastase, 12 hrs. with papain at 37°	4.7	2.9	100
	Autolyzed 24 hrs. at 37°	natural	2.3	79
	Digested 24 hrs. at 37° with takadiastase and papain simultaneously	4.7	2.6	90

*The samples of the "Average American Diet" were kindly furnished by Dr. R. R. Williams. They were prepared as described by Lane, Johnson, and R. R. Williams (16).

In general, values for pantothenic acid are highest after digestion of the sample with takadiastase; in two instances papain gives higher values. Autolysis or hot water extraction are substantially less effective, although considerable amounts are released by these methods. In some cases, e.g., beef liver and spinach, the autolytic enzymes appear able to free essentially all the pantothenic acid.

The conditions for maximum release of pantothenic acid are quite different from those obtained for riboflavin. Whereas papain digestion was found to give consistently high yields of riboflavin, the use of this enzyme for releasing pantothenic acid appears to have only occasional value. The other proteolytic enzymes also give somewhat lower results in the cases

tested. This finding is not in entire agreement with the observations of Waisman and coworkers (13) who noted higher results in some instances when trypsin digestion was employed rather than takadiastase. For assay purposes, however, it appears from the data in Tables VI and XII (p.000) that maximum release of pantothenic acid from tissues may be secured by the combined action of takadiastase and papain. Both of these enzymes are impure preparations the activities of which may vary considerably depending upon their state of purity. This may account for the discrepancy between the present data and those of the Wisconsin workers.

The results indicate clearly the presence of bound pantothenic acid, as pointed out previously by Williams (14). The ease with which these forms are broken down by the carbohydrate splitting preparations (some of which also possess high phosphatase activity) suggests the possible presence in tissue of certain pantothenic acid-ester, -amide or glucoside type linkages.

While it cannot be said with certainty whether the values obtained by enzyme treatment represent the total amounts in the tissues, it appears unlikely that any pantothenic acid remaining would be of importance from the standpoint of animal nutrition, since the animal must also rely upon enzyme digestion for extraction of the vitamin.

Support for the validity of values obtained by enzyme digestion is afforded by recent work of Willerton and Cromwell (15) who found that digestion of yeast and liver preparations with takadiastase released much bound pantothenic acid, and brought the values obtained for these materials by microbiological assays to levels approximately those obtained by chick assays.

BIOTIN

Biotin extracts were assayed by the method of Snell, Eakin and Williams (17).

The release of this vitamin from tissues is effected most completely by papain, with the other proteolytic enzymes being generally more effective than the carbohydrases. Pancreatic amylase, which probably contains some proteolytic enzymes as impurities, is also effective in freeing substantial amounts of biotin from the three tissues tested with this enzyme.

A comparison of the data in Tables I and VII shows that biotin is more firmly combined in the tissues than are any of the other B-vitamins herein discussed. The amounts which are extractable from hog heart, beef muscle, beef brain or green peas by autolysis are relatively small; furthermore, these values appear to represent only the biotin already present in the free state, since prolonged periods of autolysis fail to produce increased yields. These may represent extreme cases, for it has been shown previously (17) that autolysis of a number of materials results in greatly increased yields over those obtained by extraction with hot water. It is evident from these and other observations (2) that the effectiveness of autolysis in releasing biotin may vary greatly with different tissues.

TABLE VII

Effect of Enzyme Treatment on Yields of Biotin from Various Tissues
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Biotin Content	Relative Yield Max. 100%
Hog Heart	Autoclaved 30 minutes with 1N sulfuric acid	----	0.033	17
	Autolyzed 24 hrs. at 37°	natural	0.034	18
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	0.078	41
	malt diastase	4.5	0.036	19
	pancreatic amylase	7.0	0.19	100
	papain	5.0	0.19	100
	pepsin	2.1	0.051	27
	trypsin	8.4	0.14	74
	pancreatin	8.4	0.12	63
Beef Leg Muscle	Autolyzed 24 hrs. at 37°	natural	0.010	33
	Digested 24 hrs. at 37° with			
	takadiastase	3.0	0.022	73
	malt diastase	4.5	0.013	43
	pancreatic amylase	7.0	0.022	73
	pepsin	1.8	0.012	40
	trypsin	8.3	0.030	100
	pancreatin	8.3	0.022	73
Beef Brain	Autolyzed 24 hrs. at 37°	natural	0.025	34
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	0.056	76
	malt diastase	4.5	0.046	62
	pancreatic amylase	7.0	0.056	76
	papain	5.0	0.074	100
	pepsin	2.1	0.055	74
	trypsin	8.4	0.048	65
	pancreatin	8.4	0.043	58
Green Peas	Autolyzed 24 hrs. at 37°	natural	0.052	40
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	0.060	47
	papain	5.0	0.12	93
	trypsin	8.4	0.129	100
	Autoclaved 30 minutes with 1N sulfuric acid	----	0.11	85
Spinach	Autoclaved 30 minutes with 1N sulfuric acid	----	0.044	56
	Autolyzed 24 hrs. at 37°	natural	0.028	36
	Digested 24 hrs. at 37° with			
	papain	5.0	0.078	100
Carrots	Autoclaved 30 minutes with 1N sulfuric acid	----	0.048	100
	Autolyzed 24 hrs. at 37°	natural	0.026	54
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	0.033	69
	papain	5.0	0.048	100
	takadiastase and papain simultaneously	4.5	0.046	96
Hog Kidney	Autoclaved 30 minutes	natural	0.042	2.4
	Autoclaved 30 minutes with 1N sulfuric acid	----	0.17	9.5
	Autolyzed 24 hrs. at 37°	natural	0.098	5.5
	Digested 24 hrs. at 37° with			
	papain	5.0	1.78	100

TABLE VII—Continued

Effect of Enzyme Treatment on Yields of Biotin from Various Tissues
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Biotin Content	Relative Yield Max. 100%
Hog Leg Muscle	Autoclaved 30 minutes	natural	0.010	28
	Autoclaved 30 minutes with 1N sulfuric acid		0.020	56
	Autolyzed 24 hrs. at 37°	natural	0.013	36
	Digested 24 hrs. at 37° with papain	5.0	0.036	100
Beef Liver	Autoclaved 10 minutes		0.28	32
	Autolyzed 24 hours at 37°	natural	0.53	61
	Digested 24 hrs. at 37° with takadiastase and papain	4.5	0.87	100

Thompson, Eakin and Williams (18) have shown that extraction of biotin from tissues by sulfuric acid often reaches a maximum only after two or more hours autoclaving with 6N acid. In certain other cases Lampen

TABLE VIII

Release of Biotin from Tissues by Different Extraction Procedures

Material	Treatment	Content γ/gm.	Relative Yield Max. 100%
Beef Liver	Autolyzed at 37° for 24 hrs.	1.33	23
	Digested at 37° for 48 hrs. with takadiastase and papain	4.5	78
	Autoclaved 2 hrs. with 6N H_2SO_4	5.8	100
Beef Leg Muscle	Autolyzed at 37° for 24 hrs.	0.041	55
	Digested at 37° for 48 hrs. with takadiastase and papain	0.074	100
	Autoclaved 2 hrs. with 6N H_2SO_4	0.073	99
Beef Heart	Autolyzed at 37° for 24 hrs.	0.084	14
	Digested at 37° for 24 hrs. with takadiastase and papain	0.40	69
	Autoclaved 2 hrs. with 6N H_2SO_4	0.58	100
Blackeyed Peas, Germinated	Digested at 37° for 24 hrs. with takadiastase and papain	0.110	100
	Autoclaved 30 minutes with 1N H_2SO_4	0.097	88
	Autoclaved 2 hrs. with 6N H_2SO_4	0.099	90
Eggs	Autolyzed at 37° for 24 hrs.	0.025	14
	Digested at 37° for 24 hrs. with takadiastase and papain	0.045	26
	Autoclaved 30 minutes with 1N H_2SO_4	0.129	74
	Autoclaved 2 hrs. with 6N H_2SO_4	0.175	100
	Refluxed 6 hrs. with 6N HCl	0.157	90
Milk	Autolyzed at 37° for 24 hrs.	0.031	63
	Autoclaved for 30 minutes	0.033	68
	Digested at 37° for 24 hrs. with takadiastase and papain	0.049	100
	Autoclaved 2 hrs. with 6N H_2SO_4	0.034	69
	Refluxed 6 hrs. with 6N HCl	0.030	61
Whole Wheat Ground	Digested at 37° for 24 hrs. with takadiastase and papain	0.132	48
	Autoclaved 30 minutes with 1N H_2SO_4	0.277	100
	Autoclaved 2 hrs. with 6N H_2SO_4	0.160	58

and coworkers have found biotin to be destroyed by 2N or 4N sulfuric acid (19). Comparisons between acid hydrolysis values and those obtained by enzyme extraction are listed in Table VIII.

The data of Table VIII as well as those of Thompson and coworkers indicate that the relative yields of biotin by the two above methods are different for different tissues. In most cases, however, the discrepancy is not large.

The increasing of biotin yields by digestion with proteolytic enzymes suggests that biotin is combined with tissue proteins. The only protein-biotin complex so far known, however, is that of biotin with avidin (20) which in contrast to the complexes which occur in tissues, is not attacked by enzymes of the digestive tract.

INOSITOL

Assays for inositol were carried out by the method of Williams, Stout, Mitchell and McMahan (21).

The effects of enzymes upon the extraction of inositol from tissues are shown in Table IX.

With the four materials studied, maximum liberation of inositol is in general obtained either by hydrolysis with sulfuric acid, papain, or the carbohydrate-splitting enzymes. Autolysis yields substantial amounts of the vitamin, but only in the case of hog heart is the yield as great as those obtained by the above methods. Lower values obtained by pepsin, trypsin, or pancreatin digestion may be due to the inability of the autolytic enzymes to function at the pH values used; for when the digestions are carried out in the absence of buffers, the yields of inositol from hog heart are essentially the same with all enzymes. (See Table X.)

The data of Tables IX and X indicate that inositol complexes in tissues are for the most part rendered easily available to the test organism for growth. The close similarity of results obtained by the different methods indicates that these values represent the maximum amounts of inositol in the tissues. There remains the possibility that the increases over autolysis values, due to enzyme treatment, represent hydrolyzed phosphoric esters of the vitamin which might not be assimilable by higher organisms. The experiments of Woolley (22), however, make this supposition less likely, since phytin, soy bean cephalin and inositol hexaacetate are all active in curing alopecia in mice even though they fail to produce growth responses with yeast.

FOLIC ACID

Folic acid, like pantothenic acid and thiamin, is unstable toward the action of acid or alkali, so that comparative values do not include those obtained with these reagents. Assays of extracts were made according to the method of Mitchell and Snell (23). Since the compound has not been completely purified, assay values are computed as previously (2) on the basis of material with a "potency" of 40,000. A single homogeneous sample of Wilson's liver extract "fraction B" is taken as a standard having a potency of one.

TABLE IX

Effect of Enzyme Treatment on Yields of Inositol from Various Tissues
(γ/gm. of Fresh Tissue)

Material	Treatment	pH	Inositol Content	Relative Yield Max. 100%
Hog Heart	Autoclaved 30 minutes with 1N H_2SO_4	___	1480	93
	Autolyzed 24 hrs. at 37°	natural	1510	94
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	1550	97
	malt diastase	4.5	1600	100
	pancreatic amylase	7.0	1600	100
	papain	5.0	1600	100
	pepsin	2.1	1410	88
	trypsin	8.4	1420	88
	pancreatin	8.4	1290	81
Beef Muscle	Autclaved 30 minutes with 1N H_2SO_4	___	113	97
	Autolyzed 24 hrs. at 37°	natural	90	77
	Digested 24 hrs. at 37° with			
	papain	1.8	115	98
	malt diastase	4.5	113	97
	takadiastase	3.0	104	89
	pancreatic amylase	7.0	117	100
	trypsin	8.3	90	77
	pancreatin	8.3	94	80
Beef Brain	Autolyzed 24 hrs. at 37°	natural	1680	85
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	1750	89
	malt diastase	4.5	1700	86
	pancreatic amylase	7.0	1940	98
	papain	5.0	1980	100
	pepsin	2.1	1810	92
	trypsin	8.4	1640	83
	pancreatin	8.4	1920	97
Green Peas	Autolyzed 24 hrs. at 37°	natural	1240	83
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	1440	97
	malt diastase	4.5	1440	97
	pancreatic amylase	7.0	1360	92
	papain	5.0	1360	92
	pepsin	2.1	1490	100
	trypsin	8.4	1260	85
	pancreatin	8.4	985	66

TABLE X

Yields of Inositol from Hog Heart in Absence of Added Buffers

Enzyme	Inositol Content (γ/gm. of Fresh Tissue)	Relative Yield Max. 100%
None (autolyzed)	1120	96
Takadiastase	1170	100
Papain	1100	98
Pepsin	1100	94
Trypsin	1150	98
Pancreatin	1130	96

The differences in folic acid values obtained by different methods (Table XI) are most striking. They indicate that this nutrilite is bound

TABLE XI

Effect of Enzyme Treatment on Yields of Folic Acid from Various Tissues
(γ of "Potency" 40,000 /gm. of Fresh Tissue)

Material	Treatment	pH	Folic Acid Content	Relative Yield Max. 100%
Hog Heart	Autolyzed 24 hrs. at 37°	natural	0.068	14
	Digested 24 hrs. at 37° with			
	takadiastase	natural	0.49	100
	papain	natural	0.21	43
	pepsin	natural	0.063	12
	trypsin	natural	0.10	20
	pancreatin	natural	0.10	20
Hog Heart	Autolyzed 24 hrs. at 37°	natural	0.017	8.5
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	0.20	100
	malt diastase	4.5	0.065	33
Beef Muscle	Autolyzed 24 hrs. at 37°	natural	0.20	65
	Digested 24 hrs. at 37° with			
	takadiastase	3.0	0.31	100
	malt diastase	4.5	0.30	97
	pancreatic amylase	7.0	0.22	71
	pepsin	1.8	0.10	32
	trypsin	8.3	0.26	84
	pancreatin	8.3	0.19	61
Beef Brain	Autolyzed 12 hrs. at 37°	natural	0.11	21
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	0.52	100
	papain	5.0	0.45	87
	pepsin	2.1	0.21	40
Beef Liver	Autolyzed 24 hrs. at 37°	natural	2.5	58
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	4.3	100
	Autoclaved 30 minutes	natural	1.9	44
Green Peas	Autolyzed 24 hrs. at 37°	natural	0.63	53
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	1.2	100
	papain	5.0	1.1	92
	malt diastase	4.5	0.25	21
	pancreatic amylase	7.0	0.38	31
	takadiastase and papain simultaneously	4.5	1.2	100
	takadiastase, then 24 hrs. with papain	5.0	1.2	100
Spinach	Autolyzed 24 hrs. at 37°	natural	1.9	63
	Digested 24 hrs. at 37° with			
	takadiastase	4.0	3.0	100
	Autoclaved 30 minutes	natural	1.3	43
Carrots	Autolyzed 24 hrs. at 37°	natural	0.36	61
	Digested 24 hrs. at 37° with			
	takadiastase	4.5	0.46	78
	papain	5.0	0.26	44
	takadiastase and papain simultaneously	4.5	0.51	86
	takadiastase, then 24 hrs. with papain	4.5	0.59	100
	papain, then 24 hrs. with takadiastase	4.5	0.36	61

tightly to tissues since autolysis or autoclaving frees only a fraction of the total amount present; in hog heart approximately nine-tenths of the folic acid remains unextracted after autolysis.

Among the enzymes tested, takadiastase is uniformly superior in its ability to release folic acid from the tissues. The situation here is thus similar to those obtained for thiamin and pantothenic acid. By analogy and from the fact that papain digestion is of relatively less value in increasing yields, the presence of folic acid-ester type linkages in tissues is suggested.

In some cases papain may inactivate the autolytic enzymes present in certain tissues. Thus the yield of folic acid from carrots is less after papain digestion than after autolysis without added enzymes.

EFFECT OF TIME AND TEMPERATURE UPON ENZYME ACTION

From the data presented in the preceding sections it appears that takadiastase and papain are generally the most effective among the enzymes used in liberating the B-vitamins from tissues. It was therefore decided to perform further experiments with these enzymes to determine the optimal conditions of time and temperature for digestion.

The results are listed in Table XII. Greatest attention has been paid to the extraction of pantothenic acid, biotin, and folic acid, since the increases during enzyme digestion are greatest for these three vitamins.

Short periods of digestion with enzymes at relatively high temperatures are satisfactory in a number of cases for releasing maximum quantities of the various vitamins studied. This is especially true for cereals. Biotin, however, is not fully released by short digestion periods. Longer periods (24 hours) are to be preferred for general practice where it is desired to extract maximum amounts of the various B-vitamins.

GENERAL PROCEDURE FOR ENZYME DIGESTION

On the basis of the data thus far obtained, the following general extraction procedure has been adopted for simultaneous release of all the B-vitamins herein considered.

A one-gram sample of finely divided tissue is rinsed into a test tube with 8 ml. of acetate buffer containing approximately 1% solids and having a pH value of 4.5–4.7. To this is added 1 ml. of takadiastase suspension* (20 mg. per ml. dispersed in cold water) and 1 ml. of papain (Caroid) suspension.* The papain suspension is made by mixing equal weights of papain and glycerin into a paste and dispersing the paste in water. It contains 20 mg. papain per ml. solution. The enzyme suspension should be freshly prepared. To the 10 ml. enzyme-tissue suspension is added 0.5 ml. benzene; the sample is corked and incubated for 24 hours at 37°. After incubation the sample is treated exactly as described on page 17.

*Commercial products.

TABLE XII

Effect of Time and Temperature on Extraction of Vitamins by Takadiastase and Papain

Material	Treatment	pH	Pantothenic Acid Content γ/gm.	Pantothenic Acid Relative Yield Max. 100%	Biotin Content γ/gm.	Biotin Relative Yield Max. 100%
Beef Muscle	Autolyzed 24 hrs. at 37°	natural	4.1	80	0.015	58
	Digested 2 hrs. at 50° with takadiastase and papain	4.5	4.8	94	0.020	77
	2 hrs. at 70° with takadiastase and papain	4.5	4.4	86	0.017	65
	4 hrs. at 50° with takadiastase and papain	4.5	4.7	92	0.015	58
	24 hrs. at 37° with takadiastase and papain	4.5	5.1	100	0.024	92
	48 hrs. at 37° with takadiastase and papain	4.5	4.9	96	0.026	100
Beef Liver	Autolyzed 24 hrs. at 37°	natural	91	99	0.53	61
	Digested 2 hrs. at 50° with takadiastase and papain	4.5	82	89	0.31	36
	2 hrs. at 70° with takadiastase and papain	4.5	83	90	0.32	37
	4 hrs. at 50° with takadiastase and papain	4.5	83	90	0.52	60
	4 hrs. at 55° with takadiastase and papain	4.5	89	97	0.42	48
	6 hrs. at 55° with takadiastase and papain	4.5	92	100	0.82	94
	24 hrs. at 37° with takadiastase and papain	4.5	92	100	0.87	100
Beef Heart	Autolyzed 24 hrs. at 37°	natural	23	100	0.026	41
	Digested 6 hrs. at 55° with takadiastase and papain	4.5	19	83	0.062	99
	24 hrs. at 37° with takadiastase and papain	4.5	21	91	0.064	100
Whole Milk Powder	Autolyzed 24 hrs. at 37°	natural	13	52		
	Digested 3 hrs. at 47° with takadiastase, then 5 hrs. with papain	4.7	25	100		
	Autoclaved, then digested 3 hrs. at 47° with takadiastase, then 5 hrs. with papain	4.7	24	96		
	Digested 24 hrs. at 37° with takadiastase, then 5 hrs. with papain	4.5	24	96		
	Digested 24 hrs. at 37° with papain	5.0	23	92		
Whole Wheat Bread	Autolyzed 24 hrs. at 37°	natural	6.8	85		
	Digested 3 hrs. at 37° with takadiastase, then 3 hrs. with papain	4.7	7.9	99		
	Digested 3 hrs. at 47° with takadiastase, then 5 hrs. with papain	4.7	8.0	100		
	Digested 24 hrs. at 37° with takadiastase and papain	4.7	7.4	93		
Cornmeal (Yellow)	Autolyzed 24 hrs. at 37°	natural	2.3	82		
	Digested 3 hrs. at 37° with takadiastase, then 3 hrs. with papain	4.7	2.2	79		
	Digested 24 hrs. at 37° with takadiastase and papain	4.7	2.8	100		

TABLE XII—Continued

Effect of Time and Temperature on Extraction of Vitamins by Takadiastase and Papain

Material	Treatment	pH	Folic Acid Content γ/gm.	Folic Acid Relative Yield Max. 100%	Nicotinic Acid Content γ/gm.	Nicotinic Acid Relative Yield Max. 100%	Riboflavin Content γ/gm.	Riboflavin Relative Yield Max. 100%
Beef Liver	Autolyzed 24 hrs. at 37°	natural	8.2	92				
	Digested 2 hrs. at 50° with takadiastase and papain	4.5	7.2	82				
	2 hrs. at 70° with takadiastase and papain	4.5	6.9	78				
	4 hrs. at 50° with takadiastase and papain	4.5	7.3	83				
	4 hrs. at 55° with takadiastase and papain	4.5	7.3	83				
	6 hrs. at 55° with takadiastase and papain	4.5	6.1	69				
	24 hrs. at 37° with takadiastase and papain	4.5	8.8	100				
	48 hrs. at 37° with takadiastase and papain	4.5	8.7	99				
Whole Wheat Bread	Autolyzed 24 hrs. at 37°	natural						
	Digested 3 hrs. at 37° with takadiastase, then 3 hrs. with papain	4.7			23	61	0.96	87
	Digested 3 hrs. at 47° with takadiastase, then 5 hrs. with papain	4.7			36	95	1.08	98
	Digested 24 hrs. at 37° with takadiastase and papain	4.7			38	100	1.10	100
Cornmeal (Yellow)	Autolyzed 24 hrs. at 37°	natural			5.6	92	0.34	100
	Digested 3 hrs. at 37° with takadiastase, then 3 hrs. with papain	4.7			4.5	68		
	Digested 3 hrs. at 47° with takadiastase, then 5 hrs. with papain	4.7			6.6	100	0.32	96
	Digested 24 hrs. at 37° with takadiastase and papain	4.7			6.1	92	0.28	88

It should be borne in mind that the general effect of added enzymes upon extraction of vitamins from tissues as described in this paper is to supplement the activity of the autolytic enzymes already present rather than to replace it. Enzyme digestion of cooked materials where autolysis cannot take place will be considered in a later communication.

SUMMARY

1. The effects of various enzyme preparations upon the yields of a number of B vitamins have been determined. The most effective ones are enumerated below.

Vitamin	
Thiamin	Takadiastase and other preparations containing phosphatases.
Riboflavin	Takadiastase or papain, alone or combined; autolytic and other enzymes give variable results.
Nicotinic acid	Most effective extraction agent takadiastase or papain, alone or combined; other extraction agents often satisfactory.
Pantothenic acid	Takadiastase or combination of takadiastase and papain. Other means variable but generally less effective.
Biotin	Papain or takadiastase-papain combination; autolytic enzymes very poor.
Inositol	Papain, carbohydrases, H_2SO_4, all satisfactory. Autolytic enzymes release substantial amounts.
Folic acid	Takadiastase; autolytic, proteolytic enzymes generally poor.

2. The effects of time and temperature upon release of the vitamins by enzyme action have been studied. Short extraction periods at temperatures of 50–55° appeared satisfactory for releasing most of the vitamins studied. Biotin extractions, however, required approximately 24 hours to reach completion.
3. A general extraction procedure has been developed for simultaneous application to all of the B vitamins studied. This involves digestion with a mixture of takadiastase and papain for 24 hours at 37°.

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B VITAMIN CONTENT OF NORMAL RAT TISSUES

By

Herschel K. Mitchell and Edith R. Isbell

A previous publication from this laboratory presented values for the B vitamin contents of normal rat tissues (1). These values were based on assays of tissue extracts made after autolysis. It has since been demonstrated that this method does not free all of the combined vitamins in many biological materials (2). Consequently normal rat tissues were re-evaluated using enzymatic hydrolysis.

Tissues were taken from Wistar rats, one male and one female, chosen at random from the stock colony. The male weighed 190 grams and the female 220 grams. The animals had been on a diet of Purina dog chow.

Values for the vitamin contents are given in Table I.

From the table it may be noted that there was no significant difference between the vitamin contents of tissues from animals of different sex. Values obtained from enzymatic hydrolysis were higher in some vitamins than those from autolysis. These differences were especially significant with biotin, while folic acid, pyridoxin, and thiamin gave considerably higher values in some tissues on enzymatic hydrolysis. Muscle, lung, and heart tissue appeared the most lacking in natural autolytic enzymes as judged by the great increases in B vitamin content with hydrolysis by added enzymes.

A comparison of these vitamin levels with those of mouse and human tissues is given in a paper on B vitamins in human tissues (3) (this bulletin, p. 41).

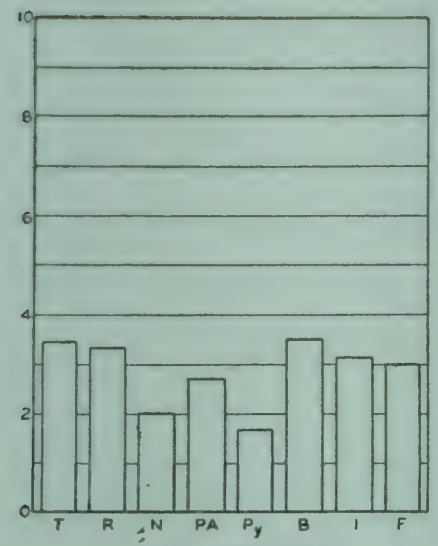
In a previous publication from this laboratory (1) "vitamin profiles" were employed as a device for comparison of a considerable number of tissues with respect to their B vitamin contents. As was pointed out at that time and further substantiated later (3) these profiles are quite characteristic for each type of tissue. In the earlier work the profiles were constructed from data based on autolysis of a whole rat as the basic unit. Since it has been found that such treatment is not adequate in some cases for freeing bound vitamins, "profiles" have been constructed using a new basic unit based on the B vitamin content of a whole rat enzymatically hydrolyzed. It is evident that a change in the values for tissue vitamins is accompanied by a roughly proportional change in the basic rat unit. As a consequence the majority of "vitamin profiles" presented in the earlier work are not radically altered by using enzymatic hydrolysis. Of the individual vitamins, biotin gives the greatest change since it is freed to a very limited extent by natural autolytic enzymes. Figure 1 gives a comparison of "profiles" obtained by autolytic and by enzymatic hydrolysis. The liver "profiles" are very nearly the same while only a

TABLE I

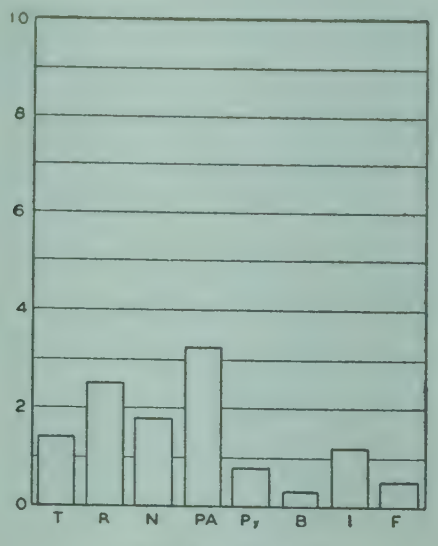
B Vitamin Content of Normal Rat Tissues

Per cent Dry Wt.	Liver 30.5		Kidney 23		Spleen 20		Heart 21		Lung 19		Brain 21		Muscle 25	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Thiamin	7.8	7.4	3.9	4.4	3.0	2.5	7.0	7.6	2.3	2.6	4.8	4.1	1.3	1.2
Riboflavin	30.	25.	29.	26.	4.6	3.0	14.	12.	3.9	4.8	2.9	3.2	1.7	1.9
Nicotinic Acid	190.	170.	110.	120.	67.	71.	120.	130.	52.	50.	64.	64.	64.	97.
Pantothenic Acid	150.	73.	32.	34.	8.5	9.5	30.	40.	9.5	8.5	12.	12.	5.5	4.5
Pyridoxin	1.6	2.1	1.0	2.2	0.35	0.43	1.1	1.7	0.29	0.28	0.88	1.0	1.1	1.3
Biotin	0.96	0.72	0.71	0.84	0.056	0.089	0.32	0.45	0.068	0.089	0.091	0.11	0.047	0.056
Inositol	640.	380.	1300.	1800.	1000.	1100.	650.	570.	960.	1100.	1800.	1500.	220.	120.
Folic Acid	6.2	5.9	7.8	8.8	6.6	5.6	2.6	3.0	4.1	3.2	3.6	2.6	1.4	1.1

Values are given in micrograms per gram of moist tissue except for folic acid, which is given in micrograms of "potency" 40,000, per gram of moist tissue.



RAT HEART
(ENZYME DIGESTED)



RAT HEART
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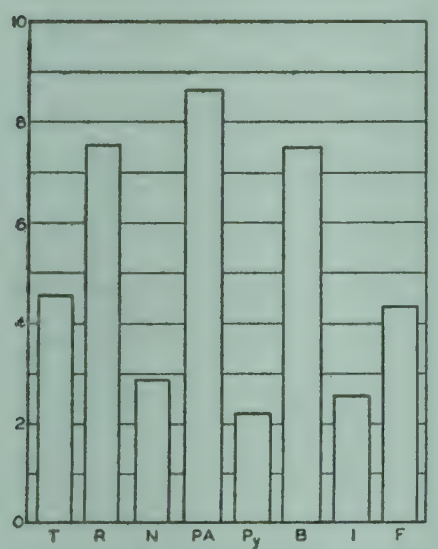
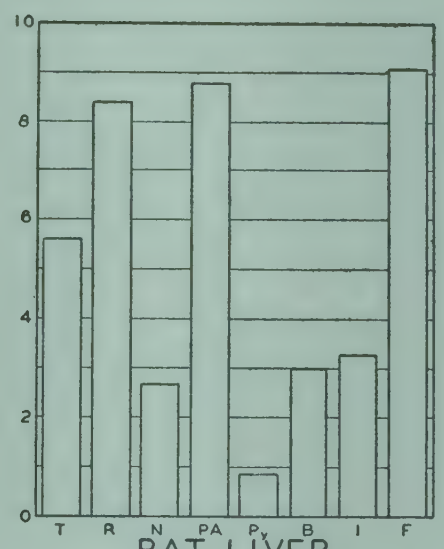
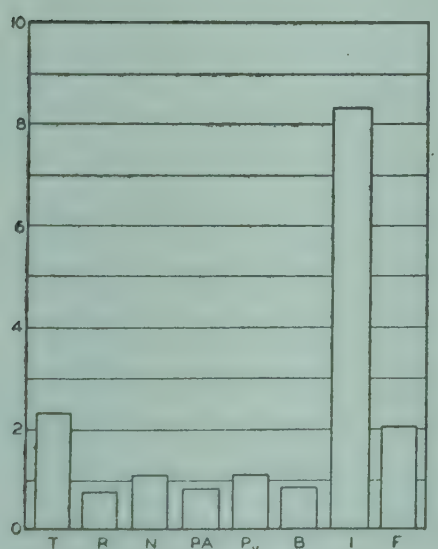


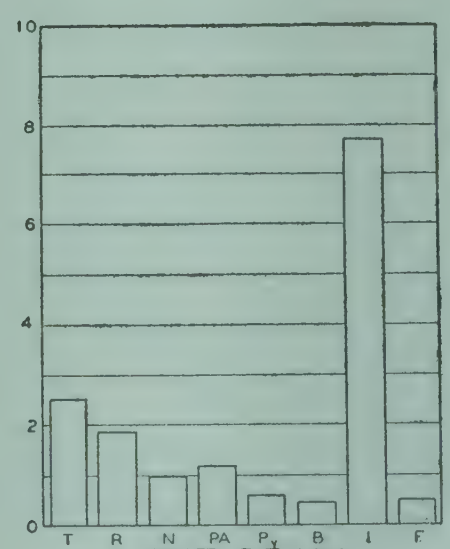
RAT LIVER
(ENZYME DIGESTED)



RAT LIVER
(AUTOLYZED)



RAT BRAIN
(ENZYME DIGESTED)



RAT BRAIN
(AUTOLYZED)

Fig. 1. "Vitamin Profiles." Normal Rat Tissues.

moderate difference is shown with the brain "profiles." A tissue such as heart muscle, which autolyzes only slightly, shows the greatest apparent change in vitamin content following enzymatic hydrolysis and thus the greatest difference in the vitamin "profiles."

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B VITAMINS IN NORMAL HUMAN TISSUES

By

Alfred Taylor, Maxwell A. Pollack, and Roger J. Williams

One of the most important series of assays which has been carried out in this laboratory has had to do with the distribution of "B vitamins" in human tissues. We are grateful to Dr. A. C. Ivy, of Northwestern University, and Dr. S. A. Levinson, of Cook County Hospital, Chicago, for their kind coöperation in furnishing us with samples of fresh tissues from the bodies of three individuals who had suffered sudden death while in apparently a normal state of nutrition. The first was a 42-year-old Mexican, weight, 160 lbs., who died from a stab wound; the second was a woman 35 years old, white, who had been found on the street in a state of collapse or death, with the exact cause of death undetermined (no indications of poisoning); and the third was a man, 24, who died from a gunshot wound.

The tissues were excised, packed in dry ice, and shipped to Austin, where they arrived in good condition. They were kept at dry ice temperature until extracts were prepared. The extracts were obtained by enzymatic hydrolysis of the samples, in the manner described in a previous paper (1) (this bulletin, p. 15).

The analyses for the different vitamins were carried out according to the microbiological methods described (this bulletin, p. 7), and the results are summarized in Table I. It should be noted that the figures are given in terms of micrograms of the vitamins per gram of moist tissue. These values may be converted to micrograms per gram of dry tissue by the use of the values for per cent solids also listed in the table. The high solids content found for the mammary gland tissue (sample 265) was due to the presence of much fat.

Solid contents which are suitable for comparative purposes were determined simply by weighing out small portions of the homogenized tissues on watch glasses and heating in an oven at 110° C. overnight. After removal from the oven, the watch glasses with their contents were immediately placed in a desiccator, and weighed when cool.

The device of showing the relative vitamin contents of a tissue by a "profile" diagram (2) is here employed again, and "profiles" showing the vitamin contents of heart, brain and liver tissue from human, rat and mouse sources are presented in Figure 1. These are plotted in terms of "rat units" previously described (2), except that the "rat units" are now revised to signify the total amounts of the vitamins released by enzymatic action instead of the autolytically released vitamins which were used as

a basis in the earlier publication. The "rat units" now represent respectively, thiamin 1.7γ, riboflavin 3.6γ, nicotinic acid 61γ, pantothenic acid 13γ, pyridoxin 0.83γ, inositol 194γ, biotin 0.11γ and folic acid 1.4γ. These are the amounts found present in one gram of rat carcass (moist basis).

To assist in the evaluation of the results, they are also presented graphically in the accompanying charts, whereby the vitamin contents of the human tissues may be compared with each other and with corresponding rat and mouse tissues. Eight charts are presented, in each of which the levels of one particular vitamin in the different tissues can be compared. The portions of these diagrams relating to rat and mouse tissues are shown in solid or diagonal shading, respectively, while the human sections are left without shading.

TABLE I

B Vitamin Contents of Normal Human Tissues

(γ/gm. of fresh tissue)

Sample No.	Tissue	Sex	Solids Content %	Thiamin	Ribo-flavin	Nico-tinic Acid	Panto-thenic Acid	Pyri-doxin	Biotin	Inositol	Folic Acid*
254	(Myocardium Heart—Left Ventricle)	♀	20.6	3.0	7.8	41	16	0.91	0.17	450	0.91
293	(Myocardium Heart—Left Ventricle)	♂	21.8	4.5	19	41	13	0.64	0.19	520	1.1
416	(Myocardium Heart—Left Ventricle)	♂	18.7	3.6	8.3	21	18	0.59	0.17	500	1.1
255	Liver	♀	27.4	2.0	16	54	31	1.9	0.62	830	7.6
299	Liver	♂	33.0	2.5	16	67	45	3.0	0.77	680	11
420	Liver	♂	28.1	2.2	18	58	43	3.6	0.74	660	24
261	Brain—Cerebrum	♀	23.8	1.4	2.1	20	13	0.60	0.031	1600	0.74
415	Brain—Cerebrum	♂	20.2	1.8	2.8	19	16	0.68	0.085	1420	1.5
257	Lung	♀	21.4	1.5	1.6	18	5.0	0.24	0.019	400	1.3
402	Lung	♂	17.8	0.62	1.9	17	2.8	0.07	0.013	310	1.2
424	Lung	♂	19.5	1.5	1.9	24	7.7	0.39	0.050	620	2.1
258	Kidney—Renal Cortex	♀	20.3	3.6	25	33	16	0.73	0.58	860	1.8
300	Kidney—Renal Cortex	♂	19.3	2.4	20	49	19	1.4	0.67	1240	2.3
422	Kidney—Renal Cortex	♂	21.5	2.8	13	37	25	1.7	0.91	1280	2.2
259	Spleen	♀	22.7	1.1	3.0	30	5.4	0.12	0.040	350	2.6
401	Spleen	♂	22.4	1.3	7.2	22	4.8	0.06	0.057	1190	3.4
419	Spleen	♂	20.9	1.1	3.6	23	6.4	0.36	0.065	1030	3.6
260	Muscle—Skeletal	♀	26.0	0.84	2.0	50	10	1.1	0.021	450	0.66
403	Muscle—Skeletal	♂	24.7	1.5	2.9	47	18	0.72	0.035	200	0.78
417	Muscle—Skeletal	♂	23.0	1.2	1.8	28	12	0.91	0.039	610	0.66
264	Muscle—Smooth	♀	22.1	1.2	2.3	31	6.2	0.53	0.058	580	0.97
262	Adrenal Gland (Whole)	♀	36.6	1.6	8.2	31	8.0	0.19	0.35	250	1.1
291	Adrenal Gland (Whole)	♂	44.3	1.7	11	24	6.1	0.14	0.23	690	1.1
414	Adrenal Gland (Whole)	♂	35.2	1.6	6.8	19	8.9	0.21	0.43	710	2.0

*Micrograms of material of "potency" 40,000.

TABLE I—Continued

B Vitamin Contents of Normal Human Tissues

(γ/gm. of fresh tissue)

Sample No.	Tissue	Sex	Solids Content %	Thiamin	Ribo-flavin	Nico-tinic Acid	Panto-thenic Acid	Pyri-doxin	Biotin	Inositol	Folic Acid*
263	Stomach—Cardiac Mucosa	♀	21.0	1.0	5.2	30	6.5	0.38	0.19	469	1.0
292	Stomach—Cardiac Mucosa	♂	20.8	0.56	5.3	19	5.6	0.18	0.11	1200	1.5
423	Stomach—Cardiac Mucosa	♂	18.4	0.36	4.4	18	6.1	0.36	0.22	761	1.2
266	Ileum	♀	17.7	1.1	2.9	29	5.3	0.34	0.064	406	1.2
294	Ileum	♂	23.1	0.43	5.0	19	3.6	0.17	0.064	867	1.6
418	Ileum	♂	19.1	0.55	4.2	14	5.6	0.27	0.094	747	1.1
267	Colon—Mucosa	♀	17.5	1.0	2.1	24	11	0.32	0.082	478	1.6
295	Colon—Mucosa	♂	32.7	1.3	2.4	13	3.9	0.21	0.092	779	2.0
421	Colon—Muscosa	♂	16.0	0.67	1.4	11	5.0	0.34	0.12	1100	2.5
265	Mammary Gland	♀	82.8	0.43	2.4	10	3.9	0.43	0.039	270	0.44
256	Ovary	♀	21.5	0.61	4.3	18	3.9	0.15	0.025	581	1.1
298	Testes—Seminiferous Tubules	♂	15.1	0.55	1.7	8.0	2.9	0.09	0.044	667	0.81
426	Testes—Seminiferous Tubules	♂	16.0	1.1	2.3	25	7.1	0.36	0.13	2600	2.3
296	Seminal Ducts	♂	19.8	0.69	1.0	9.2	2.0	0.038	0.015	<100	0.87
297	Skin	♂	44.4	0.42	1.5	8.2	2.7	0.08	0.009	<100	1.0
425	Skin	♂	37.6	0.63	0.85	9.0	3.5	0.25	0.034	297	1.0

*Micrograms of material of "potency" 40,000.

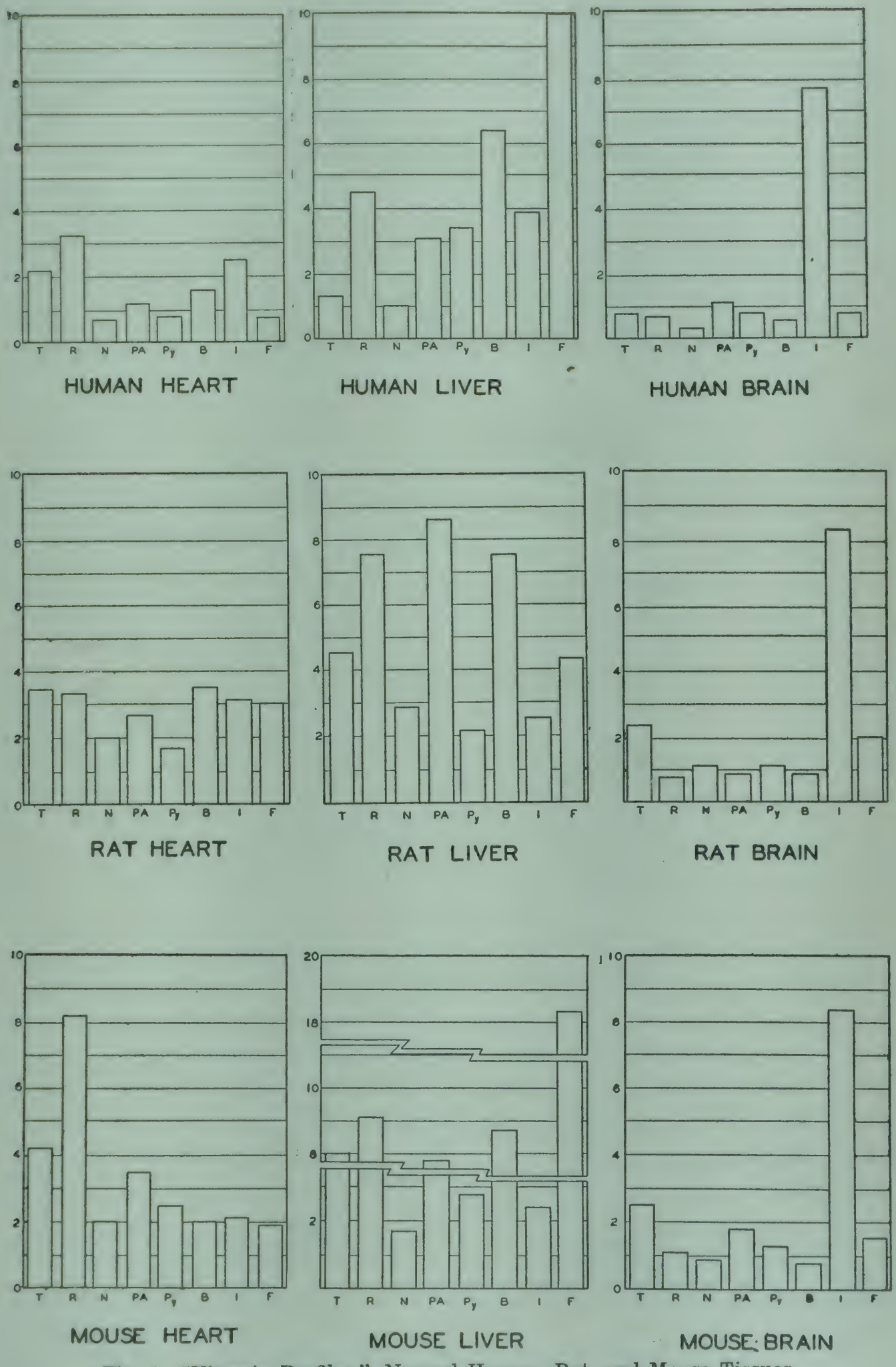

Fig. 1. "Vitamin Profiles." Normal Human, Rat, and Mouse Tissues.

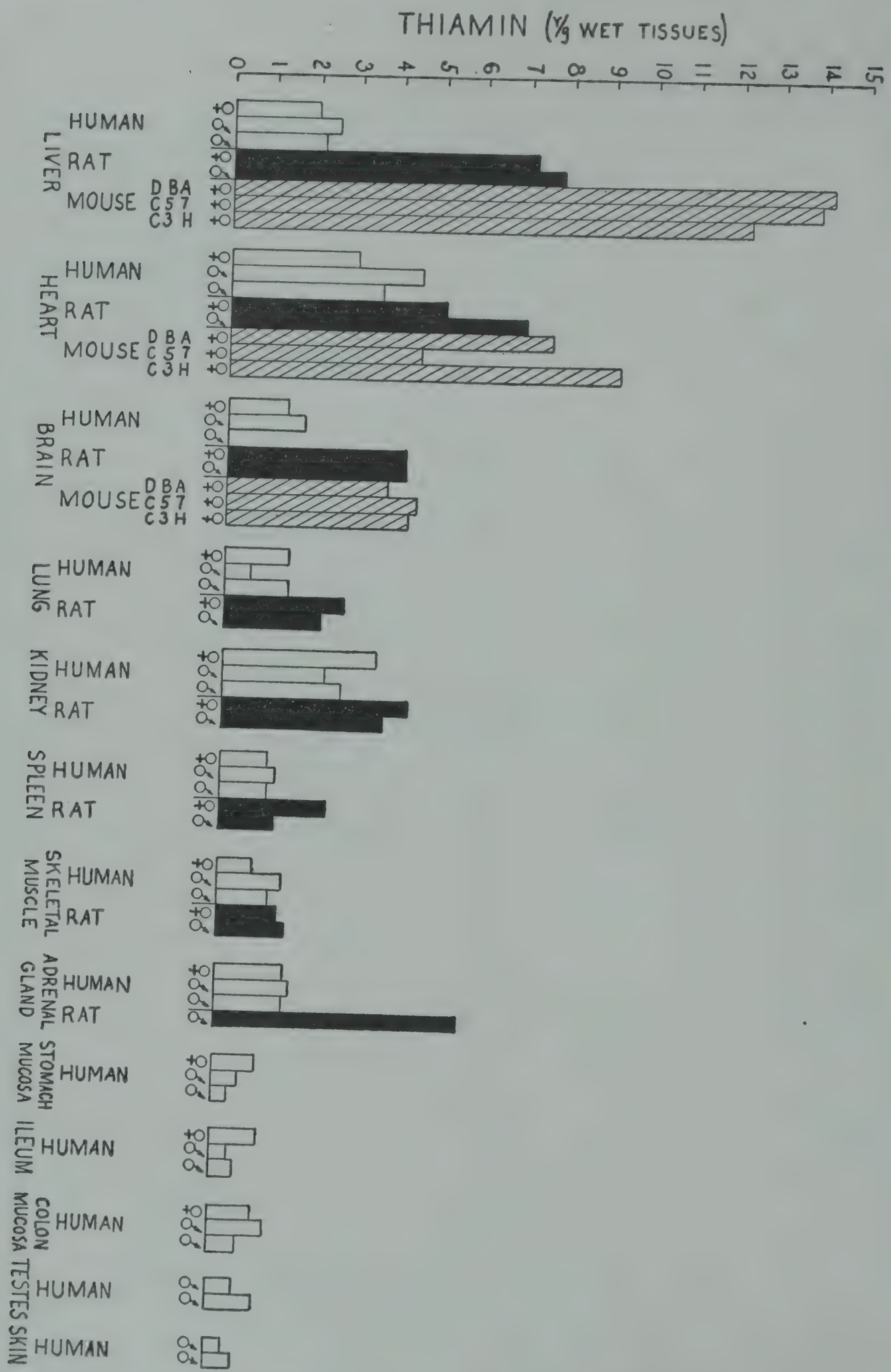

Fig. 2. Thiamin Content of Normal Tissues.

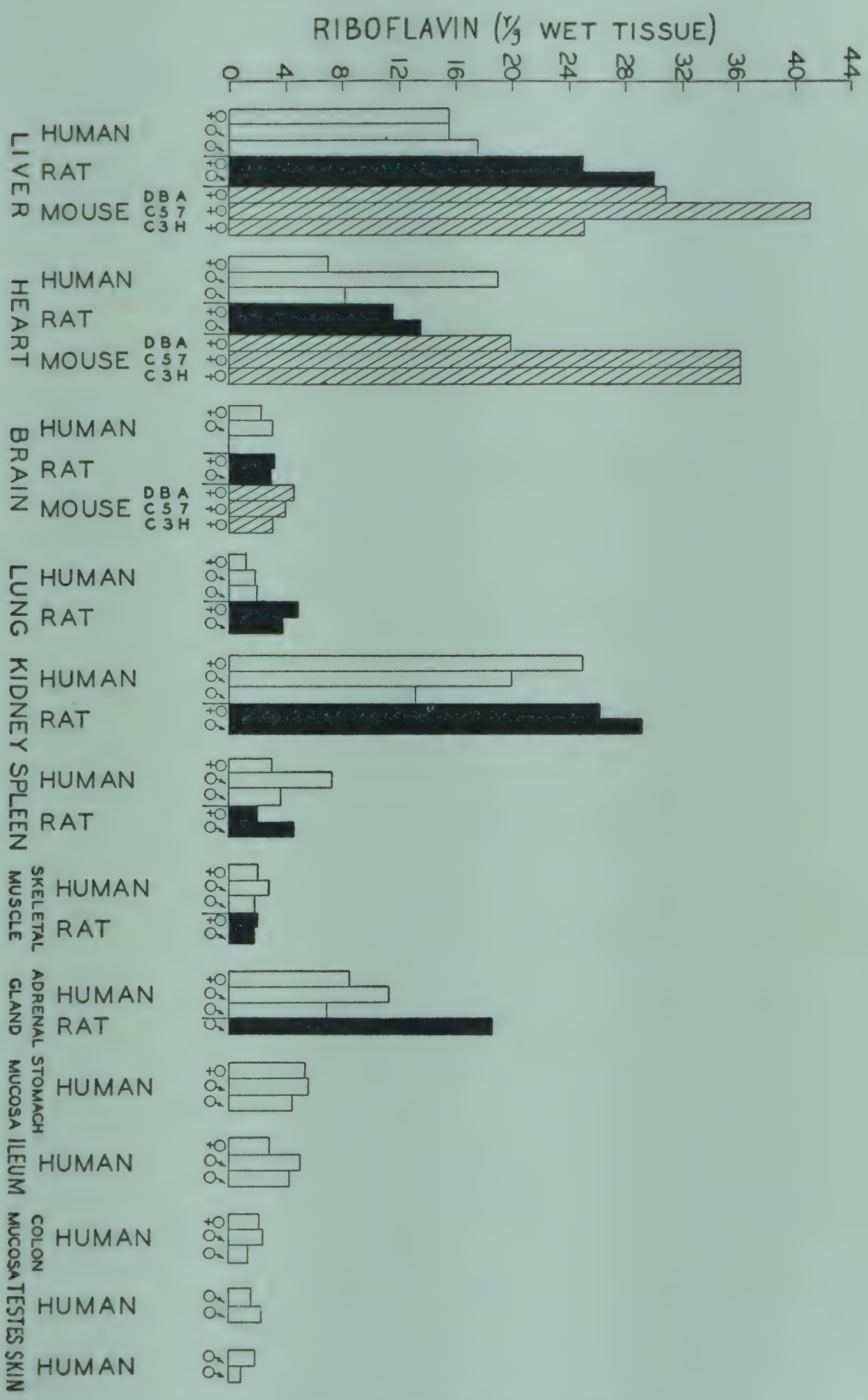

Fig. 3. Riboflavin Content of Normal Tissues.

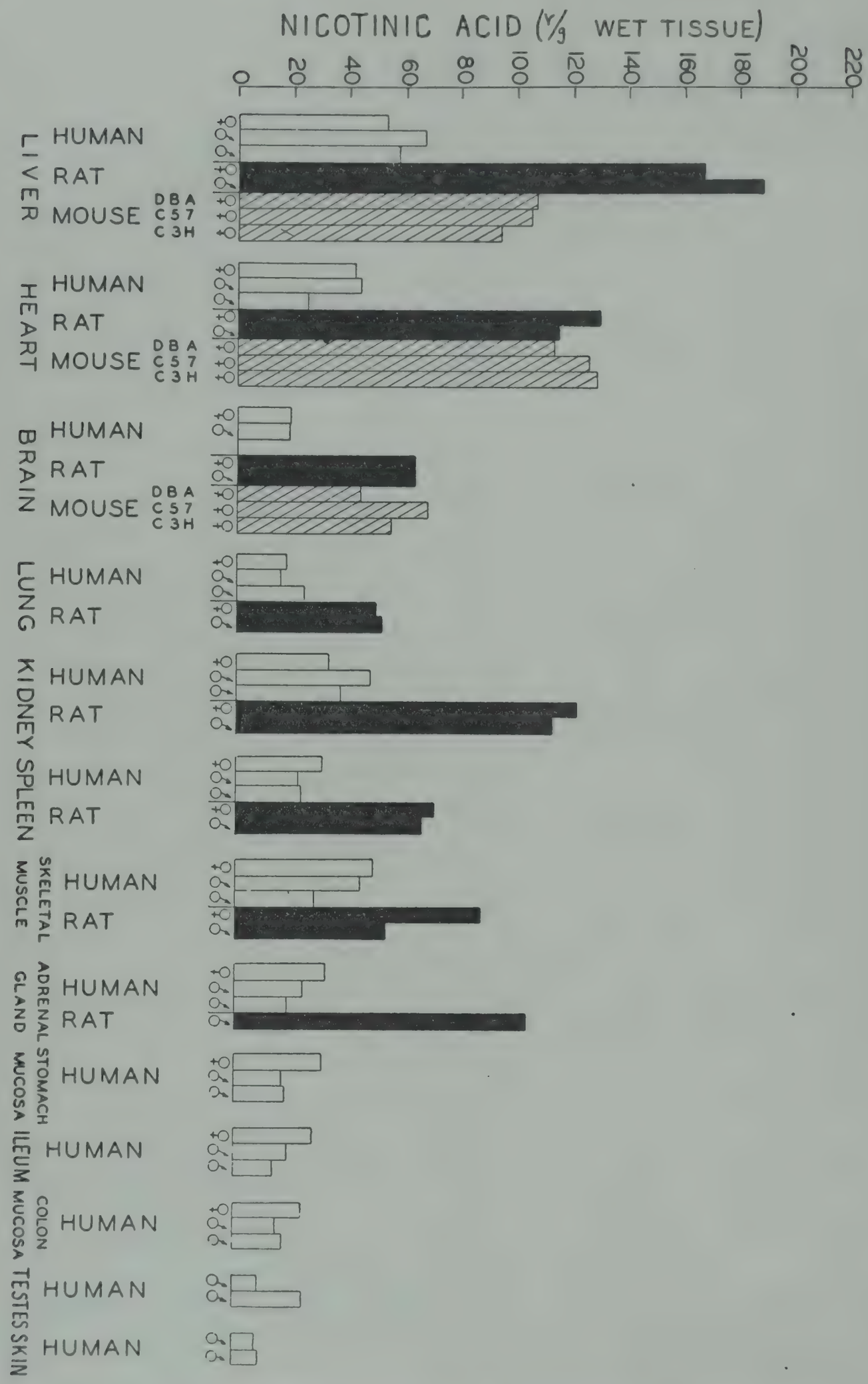

Fig. 4. Nicotinic Acid Content of Normal Tissues.

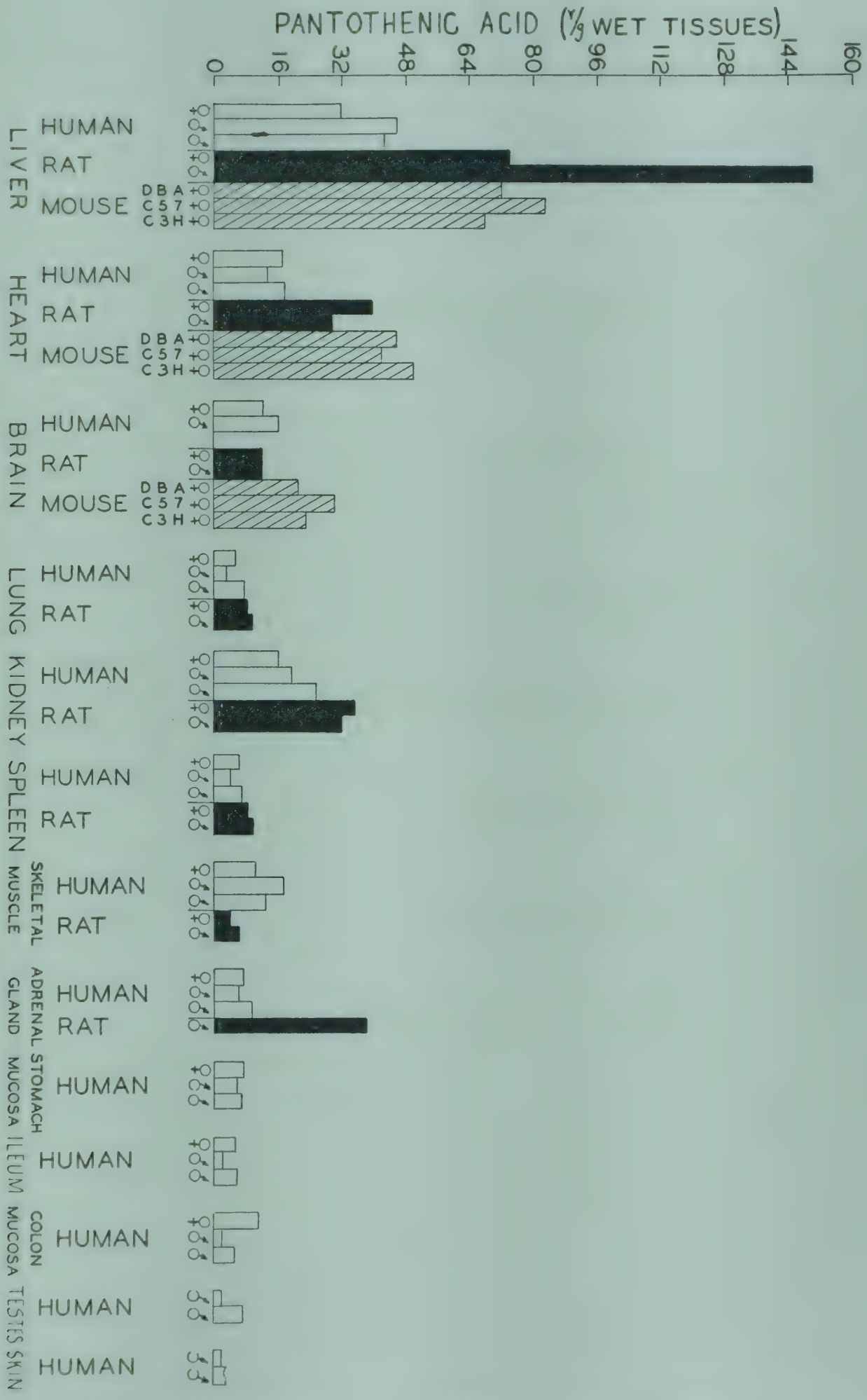

Fig. 5. Pantothenic Acid Content of Normal Tissues.

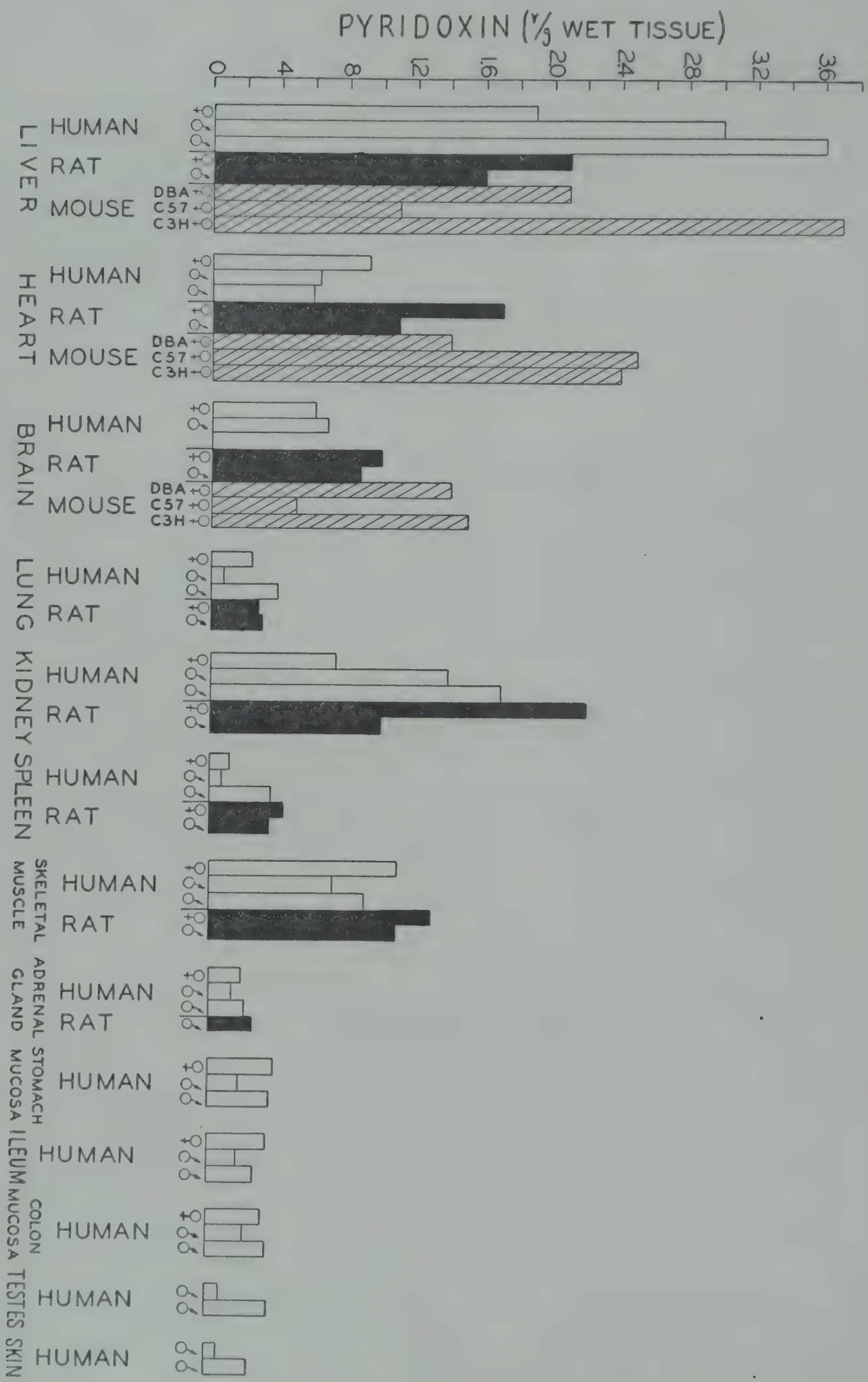

Fig. 6. Pyridoxin Content of Normal Tissues.

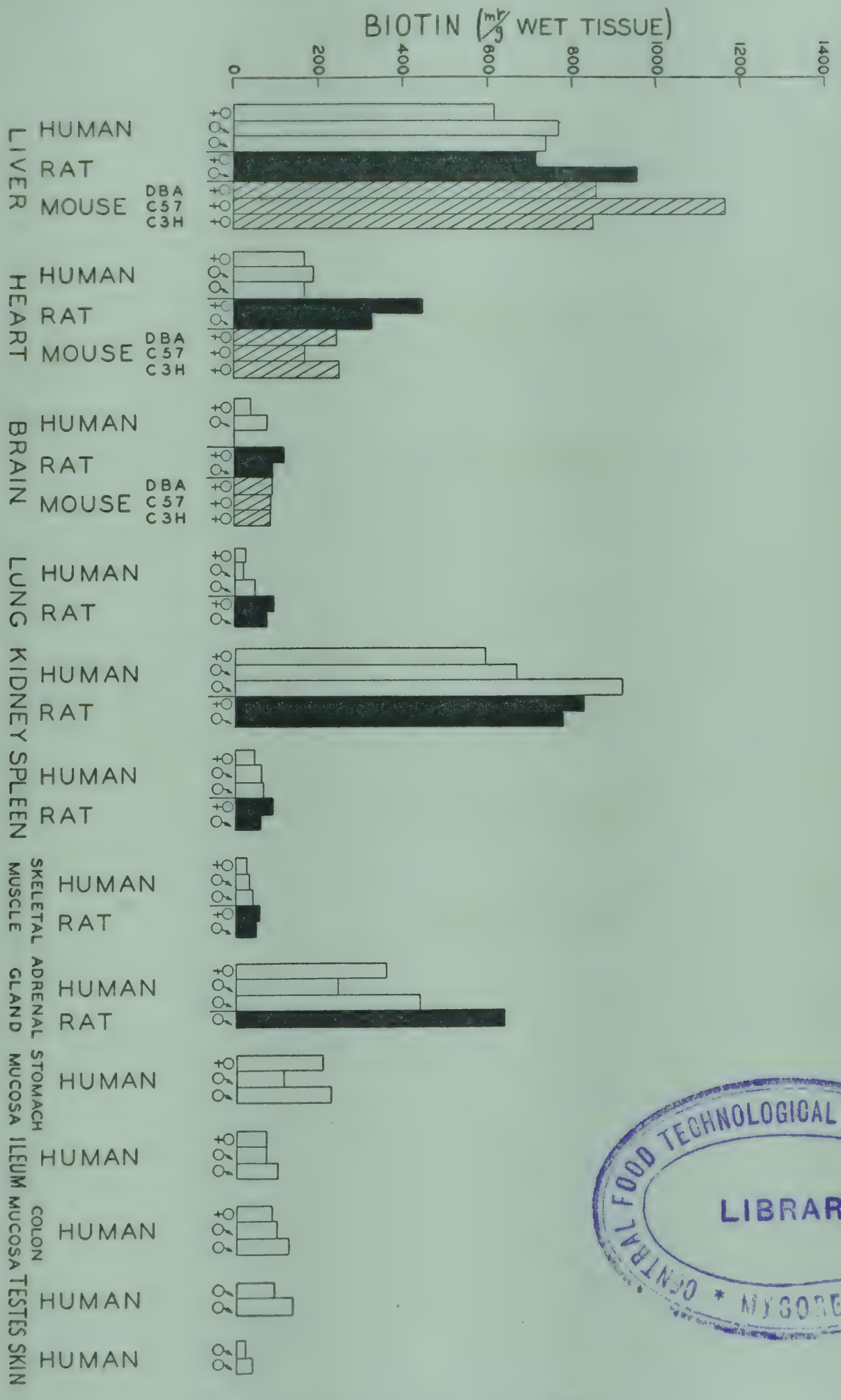

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Fig. 7. Biotin Content of Normal Tissues.

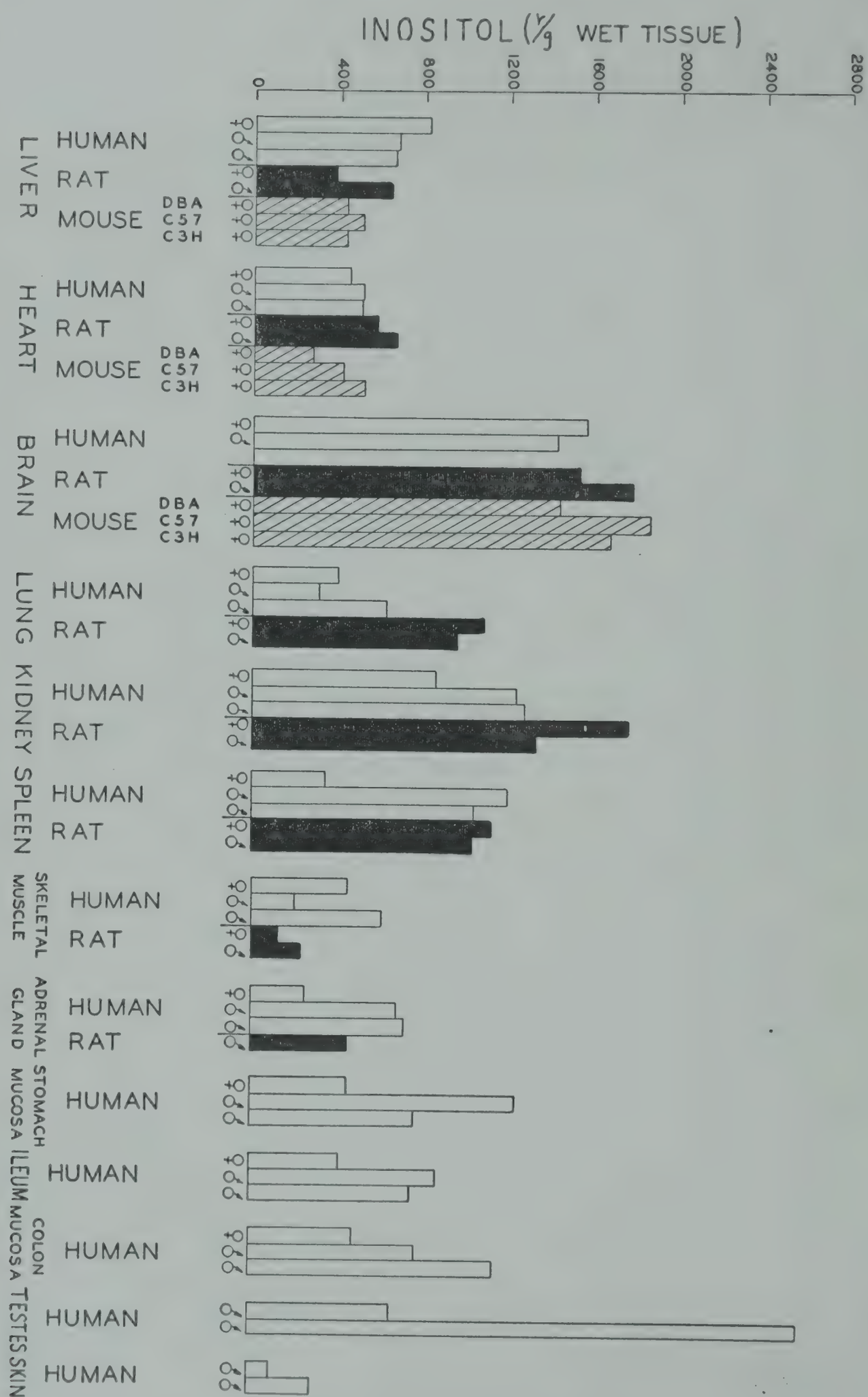

Fig. 8. Inositol Content of Normal Tissues.

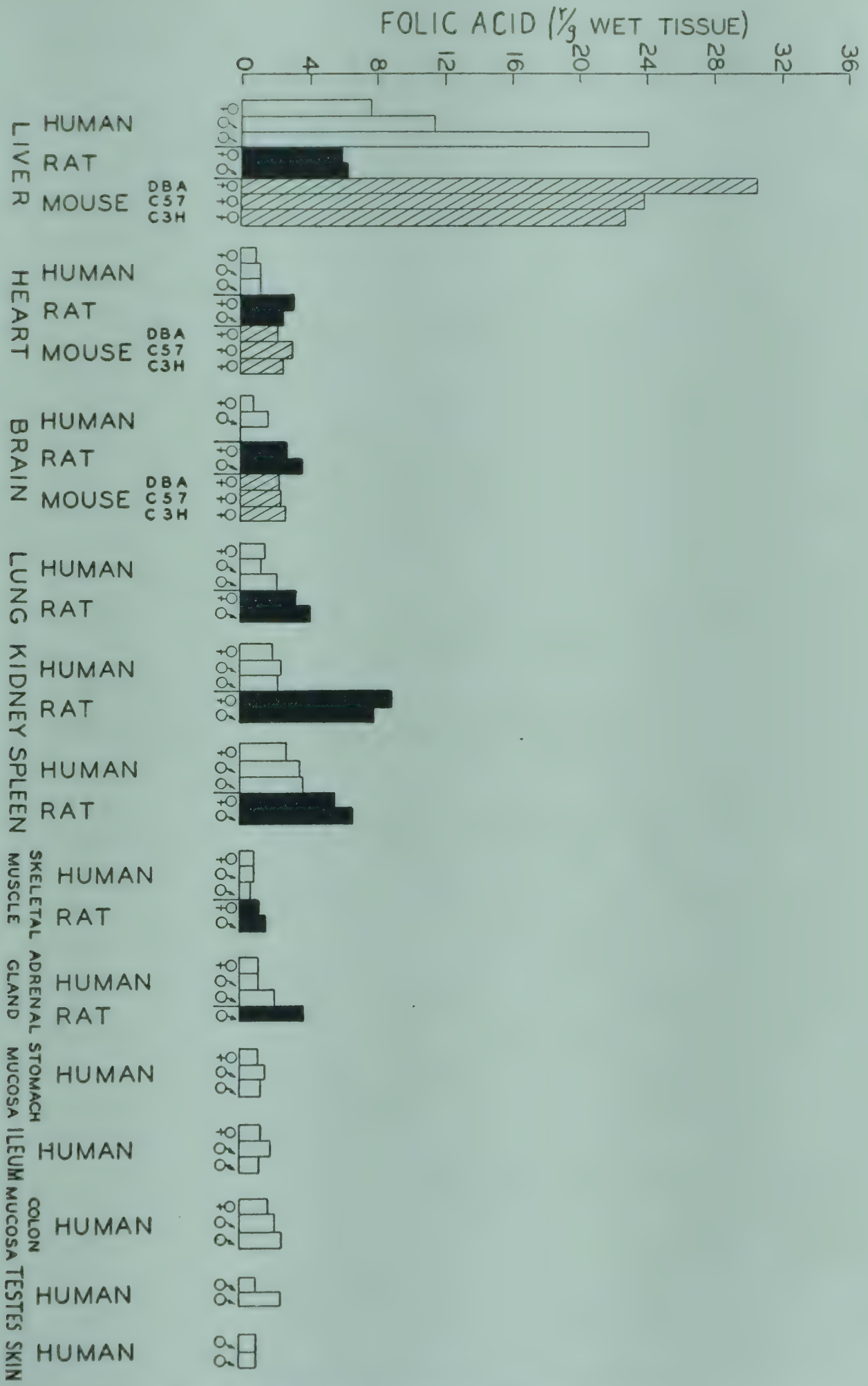

Fig. 9. Folic Acid Content of Normal Tissues.

DISCUSSION OF RESULTS

An examination of the charts (Figures 2 to 9) shows that in general the same tissues which are relatively rich sources of "B vitamins" in the rat and mouse are also rich sources compared with other human tissues.

The richest of the human tissues are as follows, in approximately the order indicated:

Thiamin—Heart, kidney, liver, adrenals.
Riboflavin—Kidney, liver, heart, adrenals.
Nicotinic Acid—Liver, skeletal muscle, heart, kidney.
Pantothenic Acid—Liver, kidney, heart, skeletal muscle.
Pyridoxin—Liver, kidney, skeletal muscle, heart.
Inositol—Brain, stomach, kidney, spleen and liver.
Biotin—Liver, kidney, adrenal, heart.
Folic Acid—Liver, spleen, kidney, colon.

Human tissues, it may be noted, are in general poorer sources of the "B vitamins" than are the rat and mouse tissues. This is in line with the species differences previously noted (2), in that there seems to be a general tendency (to which exceptions may exist) for the tissues of larger animals to be poorer in "B vitamins."

If the B vitamin contents of human and rat tissues are weighted in proportion to the amounts of the vitamins present in the whole body, we find that the differences between the vitamins of a human body and that of a rat become less in most cases and are reversed in at least one. Skeletal muscle is, of course, the most abundant tissue in each and the pantothenic acid content of human muscle appears over twice that of rat muscle. This means that the human body contains *more* pantothenic acid per kilogram of body weight than does a rat carcass. The human body appears to have somewhat the same relationship to pantothenic acid as the hog has to thiamin. In both cases other tissues in general are not especially rich, but the muscle content in both cases is high.

Riboflavin and inositol both appear to be somewhat *more* concentrated in human muscle than in rat muscle. Thiamin, nicotinic acid and pyridoxin appear almost as rich in human as in rat muscle, while biotin and folic acid are relatively low in human muscle; the latter are present in about the same proportion as they are in the other tissues.

Nicotinic acid is under physiological control in the rat, since rat tissues are capable of bringing about its synthesis. Human tissues do not possess this ability. In view of this fact it is interesting that nicotinic acid is markedly richer (usually at least twice as rich) in every rat tissue examined than in the corresponding human tissue. This is the only "B vitamin" for which this appears to be true. In the case of the other "B vitamins" some of the human tissues are at least up to the same general level as the corresponding rat tissues.

It should be pointed out that in the charts depicting the distribution of the individual vitamins in tissues (Figures 2 to 9) the indicated differences between male and female tissues may or may not be accidental, since

the graphs represent assays on individual tissues. In general the agreement between male and female tissues is such as to cause skepticism regarding real differences except where similar results have been obtained a number of times. The same may be said regarding the assay values for different strains of mice. Since individual tissues are involved, the differences may or may not be real.

More detailed knowledge regarding the functions of the individual "B vitamins" will be necessary before the peculiarities of their distribution in human tissues can be successfully interpreted.

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B VITAMINS IN HUMAN, RAT AND MOUSE NEOPLASMS

By

Maxwell A. Pollack, Alfred Taylor, and Roger J. Williams

About two years ago this laboratory became interested in the problem of the relationship of B vitamins to cancer, and it was partially for this reason that certain of the microbiological tests applicable to small amounts of tissue were developed.

The apparent universal occurrence of B vitamins in living tissue, which has been generally recognized, has made it seem more certain than ever that the B vitamins must play an important role in the peculiarities of metabolism which exist in neoplastic tissues.

We anticipated that an investigation of cancer tissues from this standpoint might yield information of various sorts. Some of the questions which we sought to answer were the following: Do cancers differ from each other as a result of such factors as tissue of origin, body site, or manner of induction? Is there a basis for the idea sometimes expressed that human and other animal cancers are fundamentally different, or is the host species a minor factor in this regard? Is there some particular vitamin (or vitamins) present in cancer tissue in relatively deficient or excess amounts?

Obviously, in order to answer some of these questions, it was necessary to have considerable background material with respect to the quantitative distribution of B vitamins in normal as well as neoplastic tissues. Such material has been collected as a result of extensive studies.

MATERIALS AND METHODS

The human cancer samples were obtained through the kind coöperation of Dr. C. P. Rhoads, of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. These tissues were packed in dry ice and sent to Austin, where they arrived in good condition. Each tissue was examined and a histological study made, the results of which are summarized below and in Table I.

These tissues cover a wide range of cancer types, and the samples always included some non-neoplastic material, as is practically unavoidable with spontaneous tumors.

The rat and mouse cancers were grown in animals of our own colony which were fed Purina dog chow—hereafter referred to as Purina. The mammary gland adenocarcinoma used in the dba mice was a transplant which had originated spontaneously in one of our dba mice and had undergone many transplantations. The corresponding cancer used in the C3H strain had been received from Dr. L. C. Turner of the National Cancer Institute, Bethesda, Maryland, and had also undergone several transplantations before analysis. The dba mouse melanoma was a transplant of one

which had been received from Bar Harbor, Maine, and the sarcomas in the C57 black mice were induced by the subcutaneous injections of a solution of methylcholanthrene in olive oil.

The rat hepatomas were induced by feeding albino rats of the Wistar strain a 3% solution of butter yellow (p-dimethylaminoazobenzene) in olive oil mixed with a basal diet of either cooked rice and raw carrots or Purina dog chow. When these hepatomas were removed, portions of the adjacent, normal-appearing livers were also removed and analyzed for comparison with the hepatomas. In some cases where the cancer was diffused throughout the liver in small deposits, the whole tissue was taken and recorded as mixed cancerous and non-cancerous liver.

The Walker 256 rat carcinoma was a transplant of one which had been received from Dr. R. E. Gardner, Johns Hopkins University. The rat mammary gland benign tumor was one which had originated spontaneously in a female albino rat in our colony, and which did not undergo successful transplantation.

Of the rat and mouse tumors, the transplants were fairly pure cultures of cancer cells, but the spontaneous and chemically-induced cancers contained more non-cancerous material.

All tissues were digested according to the procedure of Cheldelin, Eppright, Snell, and Guirard (1) (this bulletin, p. 15), and the analytical results are presented in Tables II, III, IV, and V. "Profiles" comparing the B vitamin contents of various human neoplasms with the vitamin content of a normal rat carcass are shown in Figure 1. The method of comparison used has been described by Taylor, Pollack, and Williams (2).

HISTOLOGICAL CHARACTERISTICS OF TUMOR SAMPLES

Rat and Mouse Neoplasms

It is well known that tumors even of the same type and derived from the same tissue may differ markedly with respect to such factors as the amount of non-neoplastic tissue (stroma) present and in the denseness or compactness of the cancer cells. Such characteristics are of special importance in evaluating the results of vitamin assays of tumor tissue. Some estimate of the proportion of a tumor sample occupied by neoplastic cells is especially pertinent in this regard. With these considerations in mind a study was made of each of the tumors reported in Table II.

Samples No. 107, 111, and 279 were liver tissues from rats which had been ingesting butter yellow. These samples consisted mainly of non-neoplastic parenchymal cells with scattered lesions, some of which appeared neoplastic in character. Samples 107 and 111 were from female rats which had been on the rice-carrot-butter yellow diet for a period of 134 days. Sample 279 was from a female rat which had been on Purina and butter yellow for 290 days.

Samples 169, 197, and 280 appeared to fall in the classification "hepatoma type II" after the terminology used by Edwards and White (3).

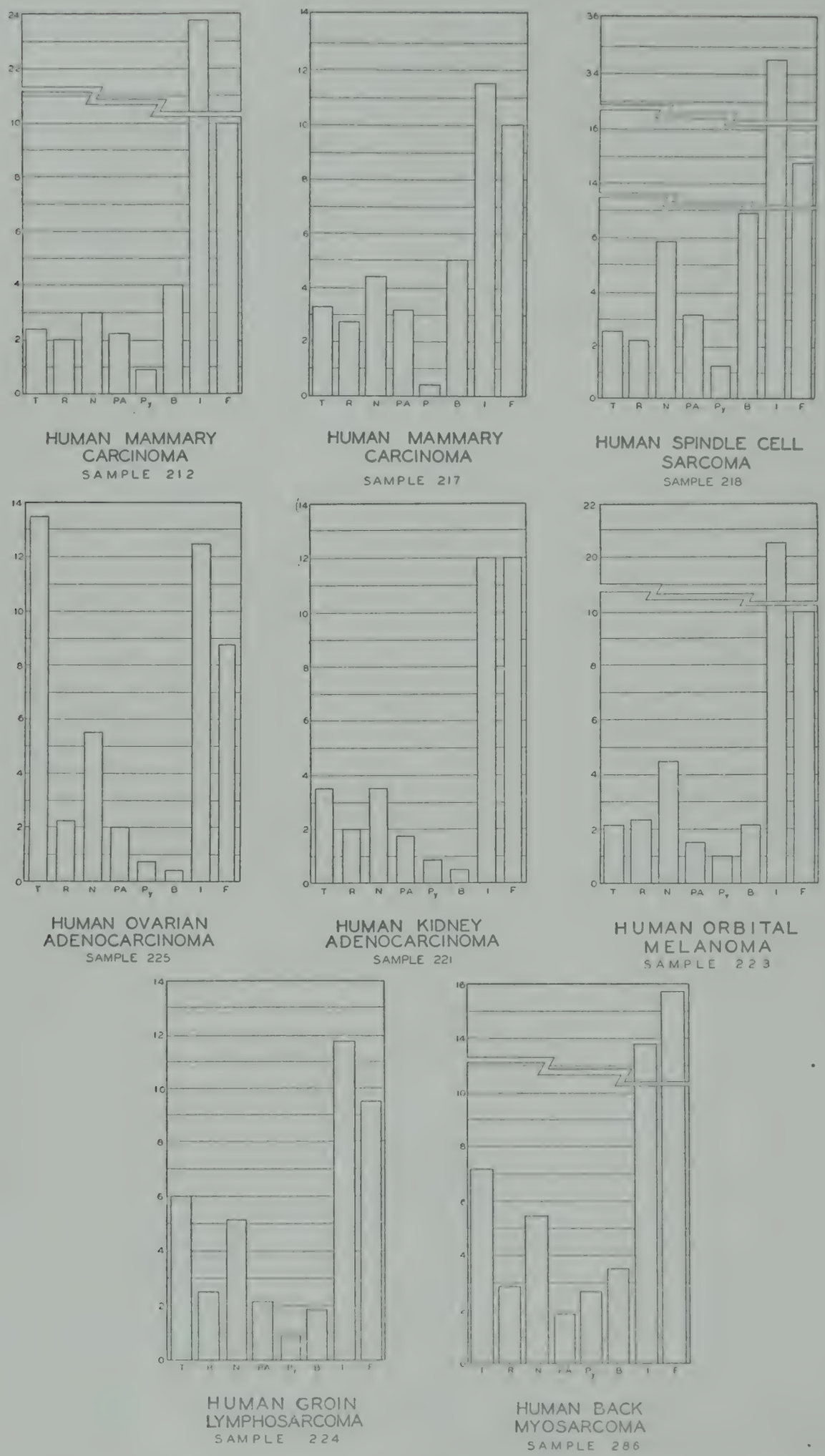

Fig. 1. "Vitamin Profiles." Human Neoplasms.

Sinuses were a prominent feature. Neoplastic cells accounted for most of the total tissue volume. Sample 169 was from a male rat which had been on a rice-carrot-butter yellow diet for 199 days, sample 197 from a female rat which had been on the Purina-butter yellow diet for 210 days, sample 280 from a female rat which had been on the Purina-butter yellow diet for 290 days.

Samples 158, 159, and 160 consisted of tumor tissues from each of three rats bearing transplants of the Walker carcinoma 256. This tumor material was of a fast growing type and was made up chiefly of neoplastic cells with a relatively scanty stroma.

Samples 207, 208, and 209 consisted of transplanted mammary adenocarcinomas from each of three female dba mice. These tumors were fast growing and non-hemorrhagic. Histological examination disclosed masses of tumor cells with a very scanty stroma.

Samples 250, 251, 252, 274, and 275 were of tumor tissue obtained from each of five female C3H mice which bore mammary adenocarcinoma transplants. These tumors were slightly slower in rate of growth than the dba mammary carcinoma transplants described above, and a hemorrhagic tendency was present. Microscopic examination disclosed fairly homogeneous masses of tumor cells with a moderate stroma. These samples tended to have a slightly smaller proportion of neoplastic cells present as compared with the preceding dba tumors.

Samples 176, 180, and 184 were taken from methylcholanthrene-induced tumors grown in C57 female mice. They consisted of fast growing sarcomas which were mainly of the spindle cell type. The stroma was negligible, the bulk of the tumor being made up of neoplastic cells.

Human Neoplasms

Data on the samples of human tissue are contained in Table I.

TABLE I

Human Tumor Samples

Sample No.	Sex	Age Years	Race	Type of Tumor	Density of Stroma	Proportion of Neoplastic Tissue in Sample Rough Approximation Per cent
105	♀	52	white	mammary carcinoma	medium	30–40
106	♀	51	white	mammary carcinoma	heavy	20–30
212	♀	57	white	metastatic carcinoma (mammary origin)	light	70–80
213	♀	71	white	mammary carcinoma	heavy	20–30
214	♀	68	white	mammary carcinoma	heavy	20–30
215	♀	58	negro	mammary carcinoma	medium	30–40
216	♀	38	white	mammary carcinoma	heavy	30–40
217	♀	58	white	metastatic carcinoma (mammary origin)	light	70–80
218	♂	41	white	spindle cell sarcoma	light	70–80
219	♀	77	white	epidermoid carcinoma	heavy	30–40
220	♀	45	white	recurrent ovarian adenocarcinoma	medium	40–50
225	♀	45	white	ovarian adenocarcinoma	heavy	50–60
221	♂	41	white	renal adenocarcinoma	medium	50–60
222	♀	1	white	axillary spindle cell	heavy	40–50
223	♀	77	white	orbital cavity melanoma	medium	70–80
224	♂	41	white	groin recticulum lymphosarcoma	heavy	60–70
281	♂	73	white	right lower leg melanoma	medium	40–50
286	♂	58	white	recurrent spindle cell myosarcoma	light	70–80
287	♀	48	white	spindle cell metaplastic salivary gland sarcoma	medium	40–50
288	♂	66	white	neurogenic sarcoma	heavy	15–25
289	♀	63	white	adenoma malignum of the sigmoid colon	heavy	30–40
290	♂	57	white	rectal adenocarcinoma	light	40–50

TABLE II

B Vitamin Contents of Human Tumors

Sample No.	Tissue	Sex	Solids Content %	B Vitamins (γ per gm. of moist tissue) Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid*
105	Human Mammary Carcinoma	♀	35.7	0.19	4.3	25	6.5	0.08	0.043	20	1.2
106	Human Mammary Carcinoma	♀	27.2	0.18	1.7	27	3.0	0.05	0.036	710	0.88
212	Human Mammary Carcinoma	♀	19.2	0.58	2.0	15	5.5	0.09	0.050	1200	2.5
213	Human Mammary Carcinoma	♀	16.1	0.53	5.7	29	7.2	0.04	0.087	740	1.7
214	Human Mammary Carcinoma	♀	34.4	0.47	1.1	21	7.9	0.07	0.097	590	1.8
215	Human Mammary Carcinoma	♀	16.8	0.64	2.9	28	11	0.03	0.039	1400	2.4
216	Human Mammary Carcinoma	♀	35.3	0.48	1.7	13	6.9	0.04	0.047	550	1.8
217	Human Mammary Carcinoma	♀	18.0	0.85	2.8	22	7.9	0.04	0.062	580	2.5
218	Human Spindle Cell Sarcoma	♂	16.4	0.67	2.2	29	7.7	0.13	0.086	1700	3.7
219	Human Skin Carcinoma	♀	17.8	0.92	1.6	18	4.0	0.08	0.020	650	2.0
220	Human Ovarian Adenocarcinoma	♀	19.5	2.1	2.2	15	4.5	0.10	0.013	620	2.4
225	Human Ovarian Adenocarcinoma	♀	14.8	3.4	2.2	27	5.0	0.06	0.005	630	2.2
221	Human Kidney Adenocarcinoma	♂	19.6	0.91	2.0	18	4.4	0.08	0.008	600	3.0
222	Human Axillary Sarcoma	♀	16.7	1.2	2.1	21	9.5	0.12	0.055	2300	3.1
223	Human Orbital Melanoma	♀	14.3	0.55	2.3	23	3.7	0.10	0.027	1000	2.5
224	Human Groin Lymphosarcoma	♂	16.7	1.5	2.4	26	5.4	0.09	0.024	590	2.6
281	Human Lower Leg Melanoma	♂	18.7	0.46	1.1	11	4.5	0.10	0.030	780	2.1
286	Human Back Myosarcoma	♂	20.4	1.8	2.9	28	4.7	0.27	0.042	690	3.9
287	Human Salivary Gland Sarcoma	♀	21.5	2.0	2.8	----	----	0.19	0.057	360	1.7
288	Human Neck Sarcoma	♂	20.0	0.83	2.1	12	2.8	0.16	0.019	610	0.60
289	Human Sigmoid Adenoma Malignum	♀	17.5	0.62	2.0	24	7.3	0.06	0.051	1100	1.4
290	Human Rectal Adenocarcinoma	♂	20.0	1.0	2.5	30	6.1	0.09	0.071	1100	1.5
405	Human Rectal Cancer	—	68.5	2.1	1.5	5.1	2.0	0.15	0.038	240	2.0

*Micrograms of material of "potency" 40,000.

TABLE III

B Vitamin Contents of Cancerous and Non-Cancerous Rat Liver Tissues

Sample No.	Diet	Days on Diet	Tissue	Sex	Solids Content %	B Vitamins (γ per gm. of moist tissue) Thiamin	Ribo-flavin	Nico-tinic Acid	Panto-thenic Acid	Pyri-doxin	Biotin	Inositol	Folic Acid*
169	Butter-yellow Rice, Carrots	199	Hepatoma	♀	17.6	2.0	3.4	24	11	0.23	.075	400	3.1
107	Butter-yellow Rice, Carrots	134	Mixed Cancerous and Non-cancerous Tissue	♀	28.4	7.4	15	140	46	1.8	.39	620	5.1
111	Butter-yellow Rice, Carrots	134	Mixed Cancerous and Non-cancerous Tissue	♀	28.4	2.8	18	120	37	1.2	.40	720	6.7
170	Butter-yellow Rice, Carrots	199	Non-concerous Liver Adjacent to Hepatoma	♀	30.5	3.0	12	100	95	1.2	.51	360	5.3
197	Butter-yellow Purina	210	Hepatoma	♀	17.6	2.2	3.9	38	12	0.21	.083	310	4.4
280	Butter-yellow Purina	290	Hepatoma	♂	17.6	6.2	7.4	59	16	0.87	.20	570	6.9
279	Butter-yellow Purina	290	Mixed Cancerous and Non-cancerous Tissue	♀	28.4	15	13	83	19	1.3	.32	530	2.9
196	Butter-yellow Purina	210	Non-cancerous Liver Adjacent to Hepatoma	♀	30.5	6.6	29	130	77	1.8	.77	420	6.5
278	Butter-yellow Purina	290	Non-cancerous Liver Adjacent to Hepatoma	♂	30.5	5.9	24	150	46	3.4	.89	620	11

*Micrograms of material of "potency" 40,000.

TABLE IV

B Vitamin Content of Rat Tumors

Sample No.	Tissue	Sex	Solids Content %	B Vitamins (γ per gm. of moist tissue) Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid*
158	Rat Carcinoma—Walker 256 Transplant	♂	14.6	1.5	2.3	20	4.5	0.18	0.020	480	2.2
159	Rat Carcinoma—Walker 256 Transplant	♂	12.9	1.1	3.3	19	5.5	0.20	0.038	720	3.6
160	Rat Carcinoma—Walker 256 Transplant	♂	14.3	0.88	4.1	17	5.5	0.16	0.032	670	4.4
406	Rat Mammary Gland Benign Tumor	♀	21.2	1.2	2.0	27	14	0.30	0.21	600	1.2

*Micrograms of material of "potency" 40,000.

TABLE V

B Vitamin Contents of Mouse Tumors

Sample No.	Tissue	Sex	Solids Content %	B Vitamins (γ per gm. of moist tissue) Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid*
207	dba Mouse Mammary Adenocarcinoma Transplant	♀	16.9	1.1	3.3	18	30	0.52	0.016	470	5.8
208	dba Mouse Mammary Adenocarcinoma Transplant	♀	19.7	1.7	3.3	26	22	0.45	0.14	190	4.7
209	dba Mouse Mammary Adenocarcinoma Transplant	♀	—	0.63	3.0	17	20	0.39	0.10	250	4.5
250	C3H Mouse Mammary Adenocarcinoma Transplant	♀	—	1.7	1.7	18	37	0.41	0.18	650	5.8
251	C3H Mouse Mammary Adenocarcinoma Transplant	♀	—	1.8	1.8	24	43	0.36	0.20	690	5.5
252	C3H Mouse Mammary Adenocarcinoma Transplant	♀	—	2.2	2.9	22	36	0.42	0.17	580	4.9
254	C3H Mouse Mammary Adenocarcinoma Transplant	♀	16.8	1.8	2.3	42	47	0.87	0.14	490	5.6
275	C3H Mouse Mammary Adenocarcinoma Transplant	♀	15.9	1.4	1.8	35	31	0.85	0.14	530	7.4
176	C57 Mouse Sarcoma (Methylcholanthrene-induced)	♀	17.3	0.93	5.5	26	30	0.24	0.094	320	3.8
180	C57 Mouse Sarcoma (Methylcholanthrene-induced)	♀	—	1.9	5.6	26	12	0.19	0.051	750	4.2
184	C57 Mouse Sarcoma (Methylcholanthrene-induced)	♀	—	2.7	6.9	35	28	0.42	0.064	590	4.8
410	dba Mouse Melanoma	♀	17.3	2.5	—	57	10	0.18	0.088	360	5.0
411	dba Mouse Melanoma	♀	17.6	2.6	4.4	64	10	0.15	0.10	610	5.2
412	dba Mouse Melanoma	♀	17.6	2.2	4.2	52	9.4	0.15	0.095	620	5.7

*Micrograms of material of "potency" 40,000.

DISCUSSION OF RESULTS

In the introductory paragraphs of this paper some of the questions are given which we had in mind when this investigation was begun and which we hoped would be answered in some degree as the work progressed. Examination of the data obtained will disclose that many of these questions have been answered much more fully than we had any right to expect. It is felt that the simplest and easiest way to bring the results obtained into focus is to consider the indicated problems in sequence and consider their status in the light of our present knowledge. First, however, a brief description of the manner in which the data have been analyzed is essential to an understanding of our interpretation of the results.

An examination of Tables II, III, IV, and V discloses a similarity in the relative concentrations of eight B vitamins in all kinds of tumors from many sources. In order to best evaluate the data, a numerical measure of this likeness or "vitamin uniformity" has been adopted.

In cases where a group of tissues are under consideration this "vitamin uniformity" for a given vitamin equals 100% minus the "coefficient of variation" where the "coefficient of variation" equals the standard deviation divided by the mean. The standard deviation was obtained from the formula for ungrouped measures (4).

$$R_o = \sqrt{\frac{\Sigma(V-M)^2}{N}}$$

where R_o = standard deviation
$\Sigma(V-M)^2$ = sum of the squares of the deviation from the mean
N = number of tissues

For smaller numbers of samples, $(N-1)$ was used in the place of N in the above equation.

In order to represent the uniformity between only two tissues a system was evolved using 100% for "vitamin uniformity" for a given vitamin if the values were identical and by representing values above or below a selected standard as some fraction of 100%. Such calculations are best represented by an example.

Example: A tissue containing 5γ per gm. of riboflavin is compared with one containing 4γ per gm. Using 5γ as a standard, $\frac{4}{5} \times 100 = 80\%$. Using 4γ as a standard, $\frac{5}{4} \times 100 = 125\%$, which expressed in terms of a deviation from 100 as a fraction of 100% equals 25% or a uniformity of 75%. The "vitamin uniformity" is then obtained as an average $\frac{80+75}{2} = 77.5\%$.

Using both of the above methods the "total vitamin uniformity" of two or more tissues is represented by an average of the "vitamin uniformities" of the individual vitamins.

These methods of analysis of the data have been applied to both normal and cancerous tissues and are believed to give a valid representation for comparative purposes. It should be borne in mind that the "vitamin uniformity" simply expresses the extent to which the vitamin content of one tissue resembles that of another.

THE VITAMIN UNIFORMITY OF DIFFERENT TYPES OF MALIGNANT TUMORS

HUMAN NEOPLASMS

Tables VI and VII contain the figures on vitamin uniformity for various kinds of normal tissues and for a group of tumors which were selected from those listed in Table II because of their relatively high neoplastic tissue content (Table I).

Examination of the tables will disclose that normal tissues of different types have very little vitamin uniformity. The eight heterogeneous normal tissues given in Table VI show a vitamin uniformity of 27 per cent. Additional examples in Table VII show splenic and renal cortex tissue to have a uniformity of 33 per cent, while renal, ovarian and mammary tissue resembled each other in vitamin level only to the extent of 11 per cent. On the other hand, tissues of the same type from different animals (e.g., myocardium), when compared in this manner possessed a vitamin uniformity of about 70 per cent.

These data indicate that vitamin uniformity is an indication of tissue similarity and in view of the part the B vitamins appear to play in basic life processes, this similarity is probably of a fundamental nature. Even the meagre knowledge concerning the physiology of these vitamins which is at present available warrants the implication that vitamin uniformity or lack of such uniformity between tissues indicates uniformity or lack of uniformity in the biochemistry of their metabolism.

TABLE VI

Vitamin Uniformity in Human Normal and Cancer Tissues
(Values in Per Cent)

Tissue	Thiamin	Ribo-flavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid	Average Uniformity
8 Normal tissues*	22	9	48	38	18	0	45	33	27
8 Cancer tissues†	29	87	80	73	81	39	59	80	66

*Striated muscle
Spleen
Renal cortex
Myocardium
Mammary gland
Smooth muscle
Ovary
Testis

No.
†212 Mammary carcinoma
217 Mammary carcinoma
218 Spindle cell sarcoma
225 Ovarian carcinoma
221 Renal carcinoma
223 Orbital melanoma
224 Groin reticulum lymphosarcoma
286 Spindle cell myosarcoma

In Table VI it will be noted that the eight tumors representing seven distinct types have a vitamin similarity of 66 per cent. The same is true for the several pairs of tumors listed in Table VII.

TABLE VII

Vitamin Uniformity in Human Normal and Cancer Tissue

Tissue	Vitamin Uniformity in Per Cent
Normal myocardium from 2 hearts	71
Normal renal cortex from 2 kidneys	66
Normal adrenal tissue from 2 adrenals	69
Normal splenic and renal tissue	33
Normal renal, ovarian, and mammary tissue	11
Mammary, ovarian, and renal carcinoma, Nos. 213, 221, and 225	60
Mammary carcinomas, nos. 212 and 225	60
Mammary carcinoma and spindle cell sarcoma, Nos. 212 and 218	62
Mammary carcinoma and ovarian carcinoma, Nos. 212 and 225	69
Mammary carcinoma and renal carcinoma, Nos. 212 and 221	70

The contrast between the vitamin uniformity of three tumors (60%) and of the three tissues of origin (11%) is particularly striking.

From the evidence contained in Tables II and III it appears that tissues of the same kind tend to be alike in vitamin content while tissues of different kinds resemble each other very little in this regard.

The seven different types of human tumors show a relatively high vitamin uniformity and must be considered to resemble each other physiologically.

RAT NEOPLASMS

In Tables VIII and IX are data comparable to those considered for human normal and tumor tissues. In this instance only two types of tumors were available, but the results duplicate those discussed above.

Again there is a lack of vitamin uniformity between different kinds of tissue and high uniformity in this respect between tissue samples of the same types. In this instance, too, cancer tissues of such contrasting types

TABLE VIII

Vitamin Uniformity in Rat Normal and Cancer Tissue
(Values in Per Cent)

Tissue	Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid	Average Uniformity
8 Normal tissues*	50	15	56	0	16	12	43	46	29.7
5 Cancers†	63	82	64	54	54	44	66	74	62.8

*Liver
Lung
Heart
Spleen
Kidney
Brain
Skeletal muscle
Adrenals

No.
†158 Walker rat carcinoma
159 Walker rat carcinoma
160 Walker rat carcinoma
169 Hepatoma
197 Hepatoma

TABLE IX

Vitamin Uniformity in Rat Normal and Cancer Tissues

Tissue	Vitamin Uniformity in Per Cent
Myocardium from two hearts	75
Cerebral tissue from two brains	89
Hepatomas, Nos. 169 and 197	78
Hepatoma and normal, adjacent liver, Nos. 169 and 196	21
Hepatoma and normal, adjacent liver, Nos. 197 and 196	23

as Walker carcinoma and chemically induced hepatoma manifest a degree of vitamin uniformity which would be expected if they constituted one tissue type.

The lack of vitamin uniformity between hepatomas and adjacent non-tumerous liver tissue is especially pertinent in this regard (Table IX).

MOUSE NEOPLASMS

Data on the vitamin uniformity in mouse normal and cancer tissue are contained in Table X. Myocardium from nine hearts had a vitamin uniformity of 76%, while 12 cancers, including carcinomas, melanomas and methyl cholanthrene induced sarcomas were uniform in this respect to the extent of 58%.

TABLE X

Vitamin Uniformity in Mouse Normal and Cancer Tissues

Tissue	Thiamin	Ribo-flavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid	Average Uniformity
Myocardium 9 hearts	71	67	84	85	73	71	78	76	76
12 Cancers*	86	50	38	43	51	56	56	85	58

*3 Mammary carcinoma transplants. (dba mice.)
3 Mammary carcinoma transplants. (C3H mice.)
3 Methylcholanthrene induced sarcomas. (C57 mice.)
3 Melanomas. (dba mice.)

Here the tumor group has a lower degree of vitamin similarity than that of the heart muscle group but the uniformity is still well above that found for heterogeneous tissue types.

The combined data from the human, rat and mouse material leads to the conclusion that different types of malignant tumor as they are found in any one species of animal are similar to each other in vitamin content, indicating they belong to a common tissue type.

THE VITAMIN UNIFORMITY OF MALIGNANT TUMORS FROM DIFFERENT KINDS OF ANIMALS

HUMAN, RAT, AND MOUSE NEOPLASMS

When comparing a tissue from a rat with a tissue from a much larger animal, such as man, there are certain differences in vitamin content

which are due to the size factor. It is well known that the rate of metabolism is higher in the smaller of two homoiothermous animals. Biological time is much faster in the rat than in man and this fact is reflected in a generally higher level of the vitamins in rat tissues.

The results of this analysis are given in Tables XI, XII, and XIII. Myocardium from rat and human hearts resembled each other to the extent of 63.5 per cent in vitamin content, while human and rat tumors were 58.8 per cent alike in this respect.

TABLE XI

Vitamin Uniformity in Human and Rat Normal and Cancer Tissues

Tissue	Vitamin Uniformity in Per Cent
Myocardium, 3 human and 3 rat hearts	63.5
8 human and 5 rat cancers	58.8

TABLE XII

Vitamin Uniformity in Human and Mouse Normal and Cancer Tissues

Tissue	Vitamin Uniformity in Per Cent
Myocardium, 3 human and 3 mouse hearts	59.0
8 human and 9 mouse cancers	47.8

TABLE XIII

Vitamin Uniformity in Human, Rat, and Mouse Normal and Cancer Tissues

Tissue	Vitamin Uniformity in Per Cent
Myocardium, 3 human, 3 rat, and 3 mouse hearts	61.2
8 human, 5 rat, and 9 mouse cancers	53.3

Myocardium from human and mouse hearts had a vitamin uniformity of 59.0 per cent, while human and mouse tumors were 47.8 per cent in agreement.

Human, rat, and mouse myocardium were 61.2 per cent alike in vitamin content against 53.3 per cent for tumors from these three animal types.

Of the tumors compared between man, rat, and mouse, it is well to recall that seven of the human tumors were distinct clinical types, that the rat tumors included both transplants of spontaneous origin and liver tumors induced by the action of *p*-dimethylaminoazobenzene, and that the mouse tumors borne by three varieties of mice contained heavily pigmented melanomas, mammary carcinoma transplants and methylcholanthrene induced sarcomas. Under these circumstances a combined vitamin uniformity of 53.3 per cent indicates strongly that malignant tumors of different host species belong to the same tissue type.

MALIGNANCY AND QUANTITY CHANGES IN THE B VITAMINS

Table XIV contains a summary of the amount of the various B vitamins present in human and rat cancer tissue in relation to the average quantity found in a group of several tissues. Insufficient determinations on normal mouse tissues were available to make the same comparison for mouse neoplasms but the limited evidence obtained indicates that the trend is generally the same.

As the data show, cancer tissue is characterized by a relatively lower level for many of the B vitamins when compared with such normal tissues as are used in Table XIV. It would be possible, of course, to select normal tissues for comparison which would be much poorer in vitamin content. Another factor which must be kept in mind is the fact that the percentage of dry matter in tumors is less than that of most normal tissues. (Tables II, III, IV, V.)

TABLE XIV

B Vitamin Levels in a Group of Human and Rat Normal and Cancer Tissues (γ/gm.)

Vitamin	Human*† Normal Tissues	Human Cancer Tissues	Cancer / Normal Per Cent	Rat*† Normal Tissues	Rat Cancer Tissues	Cancer / Normal Per Cent
Thiamin	1.80	1.28	71	3.7	1.54	42
Riboflavin	8.10	2.35	29	9.6	3.4	35
Nicotinic acid	31.20	23.50	75	87.0	23.6	27
Pantothenic acid	10.30	5.54	54	20.4	7.7	32
Pyridoxin	0.52	0.11	21	0.87	0.196	22
Biotin	0.18	0.038	21	0.22	0.05	23
Inositol	632	877	138	924	516	56
Folic acid‡	1.4	2.86	200	3.7	3.54	96

*All values are expressed as micrograms per gram of moist tissue.

†The tissues used were lung, myocardium, spleen, renal cortex, cerebrum, skeletal muscle, and whole adrenal.

‡Micrograms of "potency" 40,000.

It is evident that for both human and rat cancers, pyridoxin and biotin tend to be present at relatively low levels. The vitamin present at the highest relative level for both human and rat cancers (the same seems to be true for the mouse) is folic acid. As we begin to learn something of the functions of this substance in tissue metabolism, the relatively large amount present in malignant tumors may prove to be of special significance.

SUMMARY

B vitamin determinations have been made on a series of human, rat, and mouse neoplasms. The human material consisted of 23 malignant tumors of various types and sites. The rat tumors were of the Walker carcinoma transplant and butter-yellow induced hepatoma types. The mouse tumors included mammary carcinoma transplants, methylcholanthrene-induced sarcomas, and melanoma transplants.

The vitamin content of the tumor material was analyzed from the standpoint of vitamin uniformity and comparison made with the vitamin uniformity of mixed normal tissues and groups of normal tissues of one type.

It was found that normal tissues of the same kind have a high degree of vitamin uniformity but that mixed types of normal tissues resemble each other to a lesser extent in this regard.

The tumor material was found to have a high vitamin uniformity. This was true whether the comparison was made between tumors of one host species or between tumors of all three of the animals concerned. So far as the tumor material used in this investigation could be used as the criterion, neoplasms of different tissue origins, manner of inductions, sites and animal species thus appear to be somewhat alike in cellular metabolism, forming in effect a common tissue type.

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EFFECT OF B VITAMINS IN THE DIET ON TUMOR TRANSPLANTS

By

Alfred Taylor, Maxwell A. Pollack, and Charles L. Sortomme

Considerable attention has been given to the problem of the role of vitamins in the origin and growth of cancer. Most of the investigations in this field, however, have been handicapped, since it is only recently that many of these nutrilities have been available in a pure form. Further, until the present, even such basic information as data on the levels of the vitamins in the various tissues of different animals was lacking. This was especially true of the B vitamins, the importance of which has been emphasized by the fact that these vitamins are required for the normal functioning of all or nearly all living cells.

The present and preceding collection of papers has made available for the first time not only extensive data on the concentration of the various B vitamins in normal tissues but also on the levels of these entities in neoplastic tissue (1). Hence, it is now possible to approach the problem of the role of the vitamins in cancer tissue in a systematic manner.

The present study was concerned with the effect of various levels of some of the B vitamins on the susceptibility of the mouse to tumor transplants.

MATERIALS AND METHODS

A total of 140 dba mice composed equally of males and females and of about the same age and weight were used in this investigation. The animals were divided into 7 groups of 20 each. They were placed five to a cage, and such conditions as light, temperature and aeration were the same for all the mice concerned.

The mice of group I served as controls and were maintained on a diet of Purina dog chow—hereafter referred to as Purina. The B vitamin content of this food was determined and the results are recorded in Table I.

The other groups constituted the experimental animals and were given diets of varying vitamin content, the details of which are given in Table I. Group II was fed a diet of Purina supplemented with several times the amount of riboflavin, thiamin, nicotinic acid, pantothenic acid, inositol, and pyridoxin originally present in the ration. Choline was also added to what was estimated to be thee same elevation as the B vitamins, but since no assay of Purina with respect to this substance was available, the figure in this instance is an approximation. Group III was maintained on a diet identical with that used for Group II except for the addition of 2% yeast extract. Group IV was given a diet of Purina plus addition of about 4% by weight of beef spleen extract. Group V was given a diet of Purina plus several times the amount of nicotinic acid, riboflavin, and pantothenic

TABLE I

Vitamin Content of Various Diets Used
γ/gm.

	Thiamin	Ribo-flavin	Nicotinic Acid	Panto-thenic Acid	Pyridoxin	Biotin	Inositol	Folic Acid*	Choline
Group I Purina (Control)	3.7	5.7	39	14	2.1	0.19	1600	0.71	
Group II Purina + 7 vitamins	34	45	290	74	10	0.19	4800	0.71	600
Group III Purina + 7 vitamins + yeast extract	34	45	290	80	10	0.50	4800	0.81	600
Group IV Purina + spleen extract	3.8	6.6	43	14	1.9	0.22	1700	1.4	
Group V Purina + 3 vitamins	3.7	45	290	74	2.1	0.19	1600	0.71	
Group VI Rice-Carrot	3.4	0.22	17	7.1	0.26	0.016	140	0.05	
Group VII Purina + raw egg white	3.3	7.2	32	13	1.7	0.18	1400	0.63	

*Calculated on basis of material with "potency" 40,000.

acid originally present in the Purina. Group VI was fed boiled whole rice supplemented with salts and casein to the extent where these ingredients were present to an adequate degree, and in addition each mouse received a slice of raw carrot each day. Group VII was maintained on Purina plus 20% egg white powder.

The experimental diets were carefully homogenized by use of a ball mill.

Tumor Transplantation

For tumor transplantation, a mammary carcinoma spontaneous in origin and which had become stabilized by numerous generations of transplants was selected. Mice with tumors of about 2 grams in weight were anesthetized and bled by decapitation. The tumors were aseptically removed and only those used which were free from necrosis and hemorrhagic areas. The cancer tissue thus obtained was made into a suspension by forcing it through a muslin cloth. 10 ml. of saline solution (0.8% NaCl) was added for each ml. of tumor tissue. Each animal received 0.2 ml. of the suspension or 0.02 ml. of tumor tissue subdermally in the dorsal area just posterior to the cervical vertebrae, by hypodermatic injection, using a number 18 needle. Every effort was made to keep this operation aseptic throughout.

Hemoglobin Determination

The hemoglobin concentration of the blood was measured just prior to the initiation of the experiment and at weekly intervals thereafter. Blood was taken from the tail for these readings and the hemoglobin level determined by use of the Evelyn Colorimeter according to the method described by Evelyn (2).

Tumor Measurement

The mice were observed daily and records kept of the time of appearance of each tumor. Tumor growth was recorded by measuring two ways across the tumor and using the product of these two diameters as an index of size. A weight record of each mouse was also obtained and a record kept of death dates.

RESULTS

The data are summarized in Figures 1, 2, 3, 4, and Tables I and II. It will be noted that there was no striking deviation with respect to tumor growth, or time of death of the implanted animals by any one group as compared to the others. However, some apparently valid differences with respect to tumor susceptibility are evident.

TABLE II

Effect of Diet on Hemoglobin Concentration in Tumor-Bearing Mice

	1st Week on Diet Hemoglobin Grams Per Cent	1st Week After Implant Hemoglobin Grams Per Cent	2d Week After Implant Hemoglobin Grams Per Cent	3d Week After Implant Hemoglobin Grams Per Cent
Group I Purina (Control)	16.99	14.74	10.40	5.83
Group II Purina + 7 vitamins	16.46	14.29	13.36	6.64
Group III Purina + 7 vitamins + yeast extract	17.16	14.74	13.53	6.67
Group IV Purina + spleen extract	17.95	15.67	13.04	6.09
Group V Purina + 3 vitamins	17.84	15.70	13.14	7.22
Group VI Rice-carrot	15.91	15.38	12.83	7.98
Group VII Purina + egg white	16.71	15.15	13.03	6.70

In group II (Purina plus 7 vitamins), group V (Purina plus 3 vitamins) and group VI (rice-carrot) there occurred 3 non-"takes" of the cancer implant in each. There were 2 non-"takes" in group III (Purina plus 7 vitamins plus yeast extract). All the other groups—group I (Purina), group IV (Purina plus spleen extract, and group VII (Purina plus egg white powder) gave 100% "takes."

Table II records the effect of the various procedures on the hemoglobin concentration. In the period when the mice were on the diets but before

implantation, group IV (Purina plus spleen extract) and group V (Purina plus 3 vitamins) were associated with a distinct rise in hemoglobin level, while the rice-casein-carrot diet occasioned a lower level in this respect. After the implants were made the hemoglobin of all the groups underwent a steady decline in concentration in association with the growth of the tumors. This reaction of the hemoglobin level to tumor growth has been considered in detail in a previous publication (3).

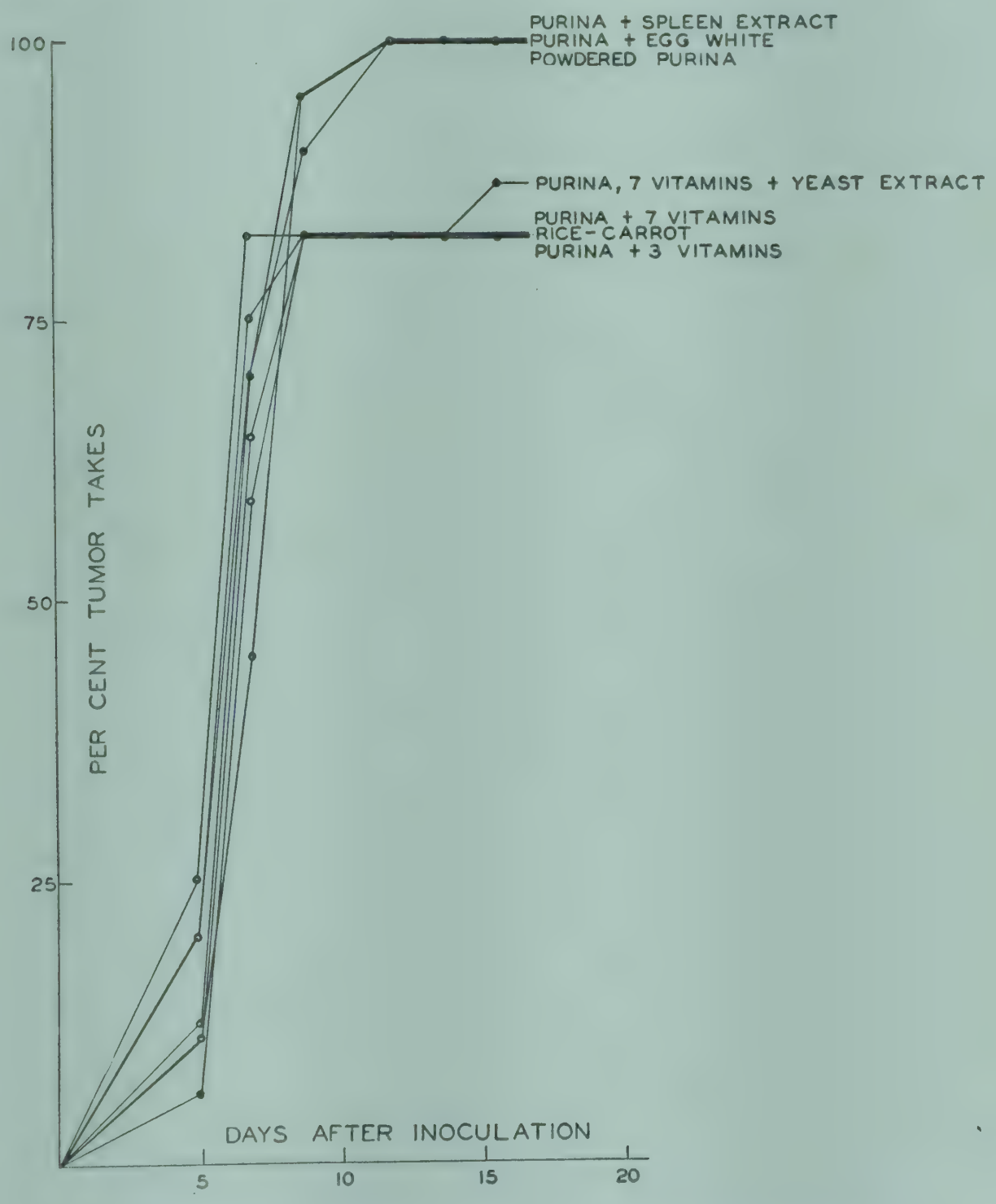

Fig. 1. Effect of Diet on Infection from Tumor Transplants among Mice.

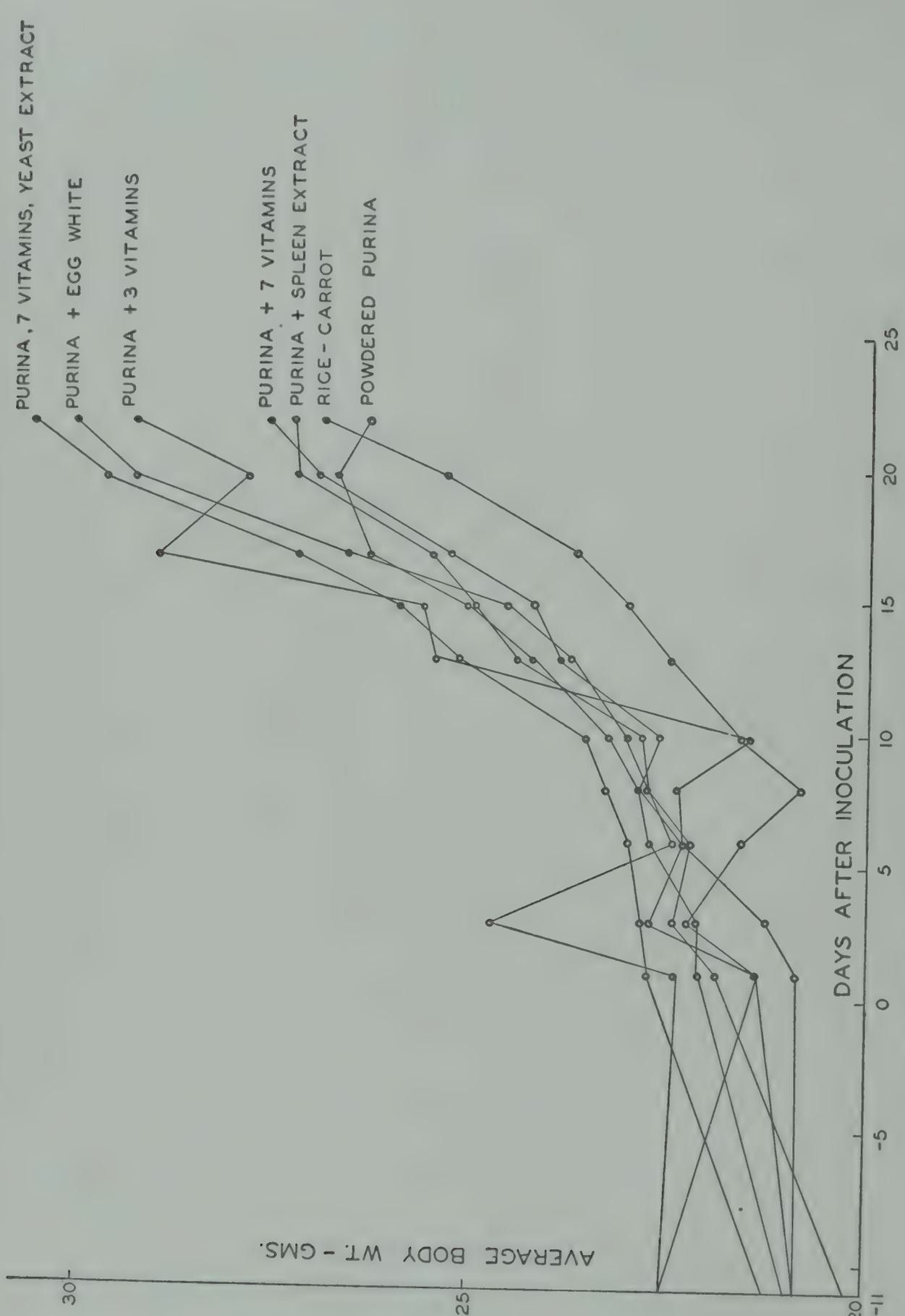

Fig. 2. Effect of Diet on Growth of Mice Bearing Transplanted Tumors.

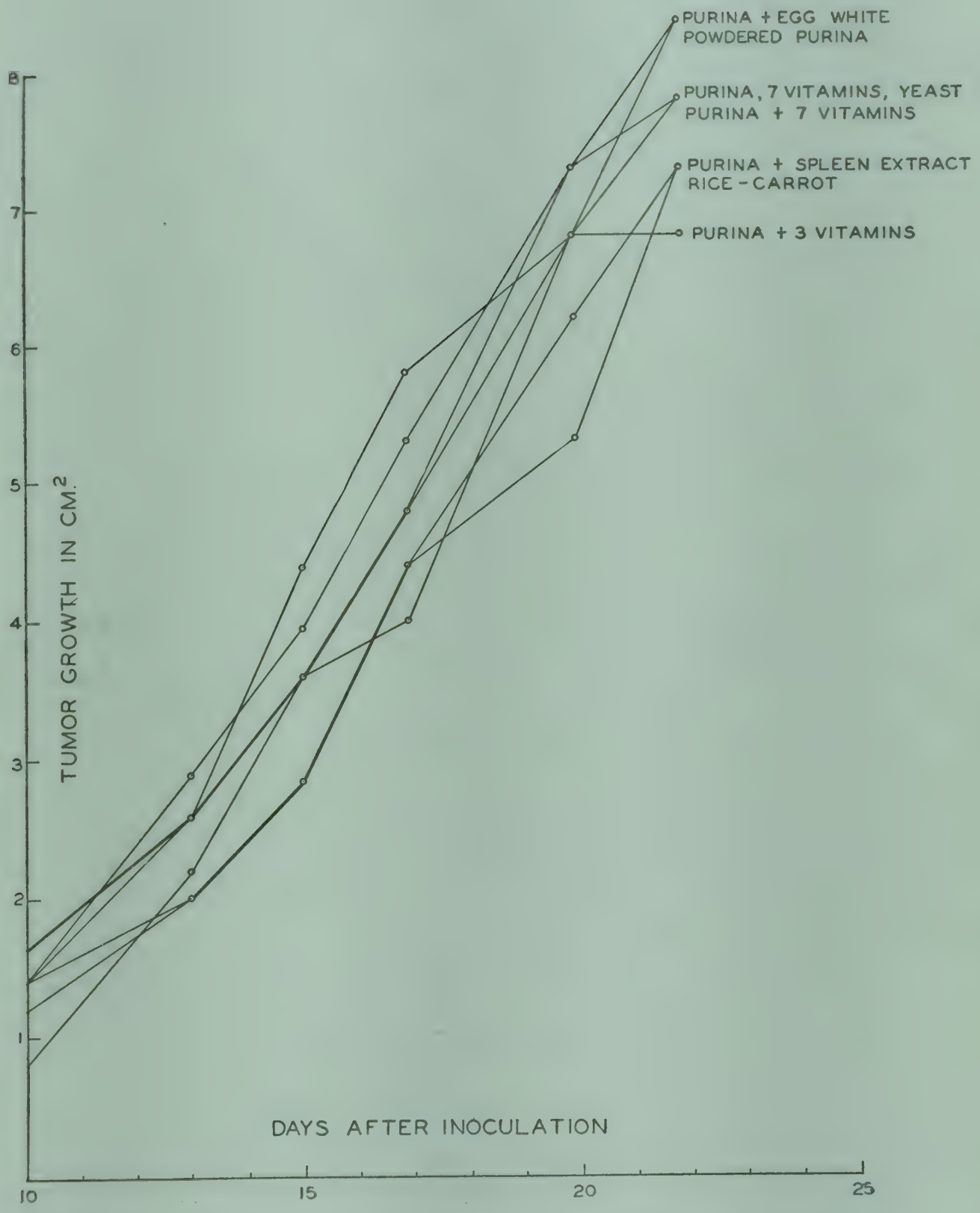

Fig. 3. Effect of Diet on Growth of Tumor Transplants in Mice.

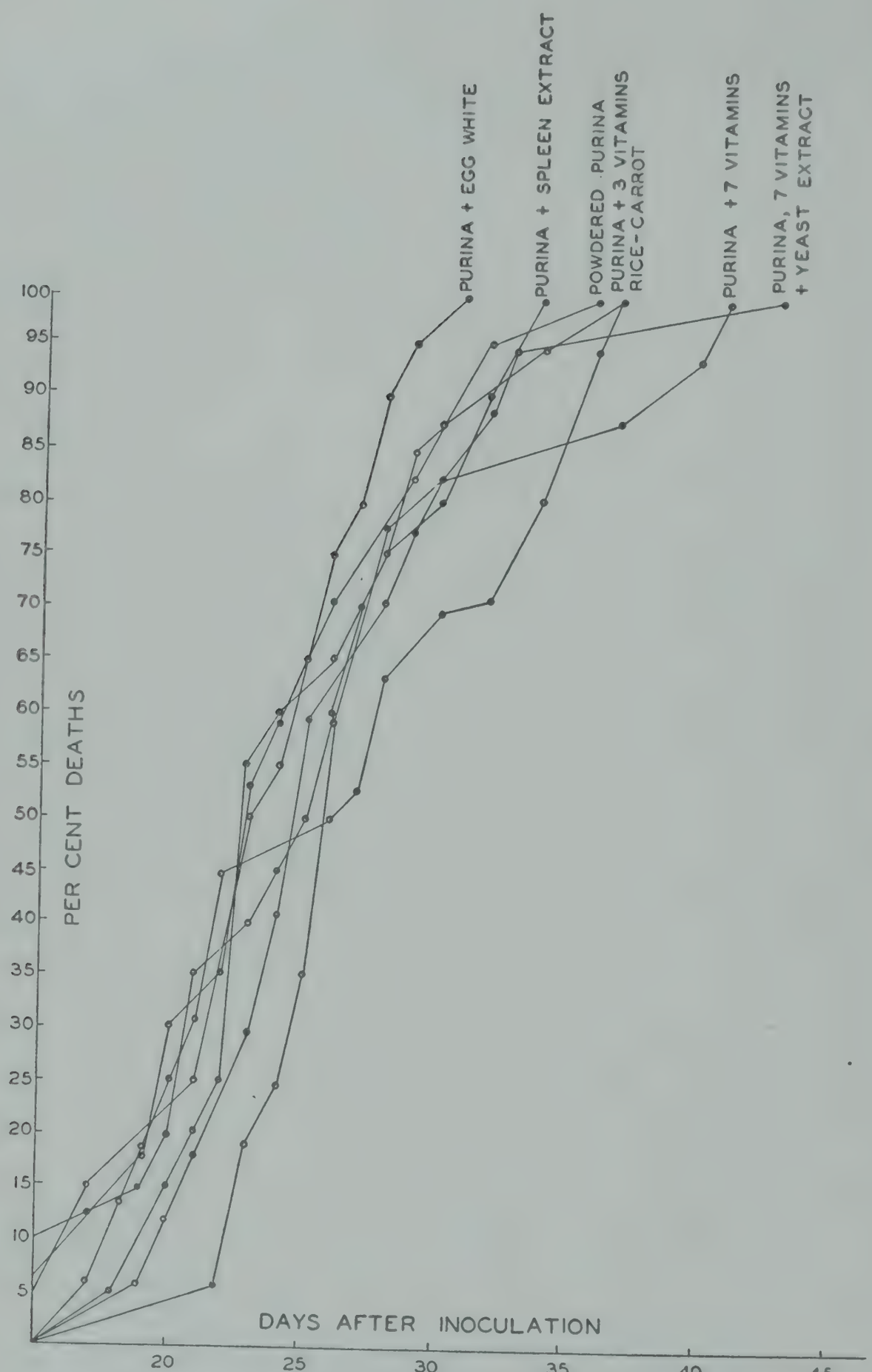

Fig. 4. Effect of Diet on Deaths from Transplanted Tumors.

DISCUSSION

It is evident that a small but definite effect on cancer "takes" was produced in the three groups receiving a diet containing several times the normal requirements of some of the B vitamins. Of these sixty mice about 13 per cent were resistant to the growth of the implanted tumor tissue. This is in contrast to the 100 per cent susceptibility shown by the 60 mice of the three groups which were maintained on diets, the vitamin content of which was approximately the same as that of Purina.

The group of 20 mice which were maintained on the rice-carrot diet also manifested resistance to the extent of 15 per cent non-"takes" to the tumor implants. The diet in this instance, as is shown by Table I, was low in most of the B vitamins, but other factors may have been responsible for the lowered tumor susceptibility.

Tannenbaum (4) has shown in a recent paper that any diet which tends to keep an animal below normal in weight will also be inimical to tumor formation and growth. In the present study this factor was not concerned, since as the data show, the animals of the various groups were approximately the same in body and tumor weight.

It is to be noted that the three groups in which no non-"takes" occurred were the ones fed diets with practically the same vitamin content. Groups IV and VII were included for other reasons. Group IV (Purina plus spleen extract) was used because of the results Lewisohn and coworkers (5) reported for this diet. There was the possibility that some vitamin which is yet unknown might be present in effective quantities in this material. We were unable to confirm Lewisohn's results. Group VII (Purina plus egg white) was used in the expectation of obtaining a biotin deficiency by this means. We have since learned that for the period of the experiment little if any biotin deficiency should be expected.

SUMMARY

A study was made of the effect of diets of different vitamin levels on the "takes" and growth of tumor transplants. 140 dba mice divided into 7 groups of 20 each were utilized.

It was found that the 3 groups which were maintained on diets in which the level of several of the B vitamins was several times greater than on Purina dog chow, 10 to 15 per cent of the mice were resistant to the growth of cancer implants as compared with complete susceptibility for the controls.

One group maintained on a low B vitamin rice-carrot diet gave 20 per cent non-"takes." In this instance, other factors may have been responsible for the changed susceptibility.

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A PRELIMINARY STUDY OF B VITAMINS IN CELL NUCLEI

By

Edith R. Isbell, Herschel K. Mitchell, Alfred Taylor, and
Roger J. Williams

In connection with studies on the function of the B vitamins in cell metabolism it appeared probable that the vitamin distribution between cells and nuclei might be an important factor. Consequently, preliminary studies on this distribution were undertaken. The problem of separation of nuclei from the cells has been approached by a number of investigators (1, 2) using two general methods:

1. Separation of nuclei from mechanically disintegrated dry tissue by fractional centrifugation from non-aqueous solvents of varied specific gravity.
2. Separation of nuclei after "hardening" in citric acid solution by fractionation and digestion of extraneous matter with enzymes.

Although the second method gives a product that is morphologically pure nuclei, it appeared probable that the vigorous chemical treatment and washing with water would remove a considerable portion of the B vitamins. This was substantiated in preliminary experiments, by preparing nuclei from the same tissues by both methods, followed by vitamin assays. The method involving the enzymatic digestion gave nuclei containing less than one-tenth the pyridoxin contained in the nuclei prepared by the other method.

The first method appeared to be particularly suitable to this problem, and it was applied to beef heart muscle and to mouse cancer tissues.

EXPERIMENTAL

Fresh beef heart tissue was ground and air dried by spreading out on paper in a current of warm air. The product was ground again and dried further in vacuum over P_2O_5. Six hundred seventy-five grams of the dried material were suspended in 4 liters of benzene and rolled in a ball mill for 1½ hours. The suspended material was allowed to settle 15 minutes and the suspension decanted from the heavier residue. This suspension was filtered and the benzene filtrate used to wash the original heavy residue by resuspension and 15 minutes settling as before. The combined residues from the benzene filtrations were again suspended in a mixture of benzene and carbon tetrachloride of sp. gr. 1.33. This material was centrifuged repeatedly at about 2,500 r.p.m. for 20-minute periods until no further precipitate was obtained. The combined precipitates were resuspended in a benzene-carbon tetrachloride mixture and the sp. gr. adjusted to 1.35. The suspension was centrifuged 20 minutes and the precipitate washed once with a solvent mixture of sp. gr. 1.35. This precipitated material contained a very high concentration of nuclei.

It was further purified by a layering process in a solvent density gradient. About 15 ml. of carbon tetrachloride were placed in a 50 ml. centrifuge tube followed by a layer of the nuclei suspended in a benzene-carbon tetrachloride mixture, sp. gr. 1.385. Fifteen ml. of the solvent mixture, sp. gr. 1.33, were placed on top and the mixture stirred gently with a glass rod to produce a uniform density gradient. The mixture was centrifuged and the top and bottom layers discarded. This fractionation was repeated twice using a smaller density range each time. The final product was dried in vacuum. Microscopic examination, after staining with methylene blue, showed it to be nearly all nuclei. The weight of the product was 242 mg. This represents about ⅛ of the yield reported by Von Behrens (2).

The low yield in this case is attributed to insufficient grinding treatment of the original dried tissue. A large proportion of the discarded material contained unbroken nucleated cells.

For preparation of nuclei from cancer tissue, the procedure was modified slightly since a much higher yield was expected. In this case the time for the preliminary grinding of the dry material in a ball mill was increased from 1½ to 5 hours. The remainder of the procedure was much the same as that described for beef heart, differing only in that less washing of the precipitates was necessary. In all cases, the concentration was followed by microscopic examination of stained tissue material.

The cancer tissue used in this experiment was obtained from cancer transplants of dba mouse mammary carcinoma, spontaneous in origin. The cancers were carefully dissected out while still small (2 gms.) before necrotic tissue had developed. One hundred grams of tissue dried to 12.0 grams. The isolation procedure yielded 3.5 grams of nuclei essentially free from extraneous benzene insoluble matter.

DISCUSSION OF RESULTS

Results of assays on the whole tissues and the nuclei are summarized in Table I. Five of the vitamins (nicotinic acid, riboflavin, pantothenic acid, thiamin, and folic acid) are two to four times as concentrated in

TABLE I*

B Vitamin Contents of Whole Tissues and Cell Nuclei

	Beef Heart		Mouse Cancer	
	Whole	Nuclei	Whole	Nuclei
Inositol	7600	2000	450	400
Nicotinic Acid	320	900	130	95
Pantothenic Acid	75	270	60	43
Riboflavin	34	130	8.3	7.0
Thiamin	32	90	9.0	7.4
Pyridoxin	4.4	4.2	0.87	0.9
Folic Acid	1.1	3.9	17	13
Biotin	0.52	0.25	0.35	0.27

*Values are given as micrograms per gram of dry material. The folic acid is given in micrograms of potency "40,000" per gram of dry material.

the dry nuclei as in the dry whole heart tissue. Inositol and biotin appear to be concentrated in the cell cytoplasm, while pyridoxin is equally distributed throughout the tissue. There is no available data on the relative weights of nuclei and cytoplasm in heart muscle, so the proportion of the total vitamins in the cytoplasm and nuclei of the living cells cannot be determined at present.

In the case of the cancer tissue all eight of the vitamins are more concentrated in the whole tissue than in the cell nuclei. Since the dry cancer tissue contains about one-third of its weight as nuclei, about three-fourths of the total cell B vitamins are contained in the cytoplasm.

No significant interpretation of the difference in distribution of vitamins between nuclei and cytoplasm of heart muscle and mouse cancer tissue can be made at present. It appears probable, however, that a further study will reveal important differences in distribution of vitamins within the tissue cells.

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THE B VITAMIN CONTENT OF ORGANISMS OF DIFFERENT BIOLOGICAL PHYLA

By

Alethea M. Woods, Jean Taylor, Margaret J. Hofer,
Gerald A. Johnson, Robert L. Lane and J. Raymond McMahan

This paper represents a survey of the B vitamin contents of a number of organisms of various phyla and divisions throughout the biological kingdom. The ubiquitous character of certain of the B vitamins has been made apparent by numerous workers, and it is now desirable to extend our studies over a wide range including all of the eight B vitamins which are being assayed in this laboratory.

Organisms have been selected at random from phyla Protozoa, Mollusca, Arthropoda, Annelida, and Chordata. In phylum Chordata representative organisms are used from classes Reptilia, Mammalia, Amphibia, Pisces, and Aves. Likewise samples have been made of several members of class Insecta. (Table I.)

In the plant kingdom divisions Thallophyta and Spermatophyta are represented. Among the higher plants assays have been made of the flower, root, tuber, leaf, seed, and fruit. (Table II.)

PROCEDURE

Extracts were prepared according to the general procedure of Cheldelin *et al.* (1) (this bulletin, p. 15). Microbiological assay methods as described earlier (this bulletin, p. 7) were employed throughout.

In Table I are given data obtained from various organisms mentioned above, as well as from many higher animals and plants.

It will be noted that all materials tested contain all of the B vitamins, although in varying proportions.

In the animal kingdom there is a tendency toward an inverse ratio between the size of organisms and the levels of the B vitamins in their tissues. This observation is in accord with others made previously (2, 3). This trend is especially notable with riboflavin, pantothenic acid and folic acid.

Among the animals and microorganisms studied, the richest source found is seldom more than ten times that of the poorest source. *Drosophila* larvae are exceptional, being about 60 times as rich in folic acid as the poorest source tested.

The insects tested (ants, cockroaches, termites, and two strains of *Drosophila* larvae) are all rather rich and relatively uniform in their content of thiamin, riboflavin, nicotinic acid, and pantothenic acid, but

TABLE I

B Vitamins in Organisms of Different Biological Phyla
(γ/gm. of moist tissue)

	Dry Wt. %	Thiamin	Ribo-flavin	Nico-tinic Acid	Panto-thenic Acid	Pyri-doxin	Biotin	Inositol	Folic Acid†
Fish *(Cryprinidae)*	30.6	2.9	1.6	23.9	7.5	1.1	0.095	270	1.64
Frog *(Rana)*	21.8	1.4	2.52	11.7	3.7	1.2	0.126	270	0.316
Snake *(Thamnophis)*	19.5	1.0	9.11	28.0	5.1	0.8	0.050	210	1.71
Chick embryo	7.0	.58	0.93	28.4	26.0	0.47	0.123	83	2.49
Red Ant *(Dolichederus)*	43.0	3.2	6.08	20.5	12.5	0.67	0.160	960	1.54
Cockroach *(Periplaneta americans)*	27.0	4.4	7.11	33.0	17.5	1.3	0.130	360	0.85
Termites *(Zootermposis)*	18.0	2.3	4.75	32.0	16.0	0.32	0.119	390	2.23
Dros. virilis larvae, N.Y.	17.1	4.2	8.11	36.5	20.0	1.3	0.352	160	11.1
Dros. virilis larvae, N.O.	19.0	4.4	8.22	37.5	20.5	0.94	0.374	250	18.6
Oyster *(Mytilus)*	16.4	1.8	2.09	11.7	4.9	0.45	0.087	440	2.26
Earthworm *(Lumbricus terrestris)*	31.6	2.5	8.0	15.0	3.2	0.29	0.079	164	0.706
Protozoa *(Tetrahymena geleii)*	13.0	5.0	2.22	11.7	13.8	3.1*	0.098	432	3.04
A. aerogenes, aerobic	20.7	2.2	9.0	49.1	30.0	1.4	0.800	280	2.8
S. marcescens	16.9	4.6	5.9	40.1	20.9	1.79	0.699	280	2.75
P. fluorescens	21.0	5.5	14.1	44.0	19.1	1.19	1.490	360	1.84
C. butylicum, anaerobic	29.0	2.7	15.9	73.0	26.9	1.79	0.490	250	0.812
Mushrooms *(Coprinus atranentarius)*	12.5	1.1	3.26	68.5	17.0	0.45	0.180	170	0.98
Brewers' Yeast	100.0	8.5	15.2	126	42.5	1.0	0.071	280	1.05
Mold	20.3	0.09	0.96	12.2	3.0	0.47	0.021	260	1.84
Horned toad	15.8	1.7	3.4	26.8	5.8	1.2	0.112	330	1.37
Rat (whole)	34.0	1.7	3.6	61.0	13.0	0.83	0.11	190	1.4
Lamb leg muscle	30.5	2.9	2.4	75.0	6.0	0.81	0.021	580	1.1
Veal leg muscle	32.0	1.8	2.2	72.0	1.1	0.56	0.020	320	0.92
Chicken leg muscle	22.3	0.78	2.6	38.0	6.2	0.25	0.098	470	1.2
Salmon steak	28.1	1.3	1.4	64.0	6.6	0.33	0.053	170	0.87
Whole Wheat (seed)	90.0	5.0	1.6	41.0	12.0	2.1	0.052	1700	1.9
Lima Beans dried (seed)	92.0	5.3	1.3	9.8	8.3	5.5	0.098	1700	3.3
Cauliflower (flower)	11.7	1.4	1.3	5.7	9.2	0.20	0.17	800	1.4
Carrots (root)	11.8	.38	0.5	2.6	2.5	1.2	0.025	480	0.97
Blackeyed Peas dry (seed)	95.0	8.1	1.4	13.0	10.4	1.9	0.21	2400	7.4
Apples (fruit)	14.5	0.96	0.18	0.81	0.6	0.26	0.009	240	0.08
Watermelon (fruit)	10.0	0.56	0.69	2.4	3.1	0.33	0.036	640	1.5
Lettuce (leaf)	5.2	0.39	0.27	2.5	1.1	0.71	0.031	550	0.38
Irish potato (tuber)	22.2	1.7	0.29	4.3	3.2	2.2	0.006	290	1.4

*Assay not satisfactory. †Micrograms of material of "potency" 40,000.

their content of the other B vitamins is variable. The two strains of *Drosophila* larvae give results in close agreement, and except in the case of folic acid, these results are similar to those of other members of that class.

The same four vitamins which are abundant in insects are likewise abundant in bacteria and yeast. The vitamin contents of fish, frog, snake, rat, and earthworm are quite uniform.

Inositol, folic acid, and pyridoxin are found frequently in greater concentration among the plants investigated than among the animal organisms tested. This is especially true when dry weights are considered. Nicotinic acid, on the other hand, is generally much richer in animal tissues.

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SYNTHESIS OF B VITAMINS BY BACTERIA IN PURE CULTURE

By

Roy C. Thompson

While many bacteria require one or more of the B vitamins for growth on a synthetic medium, it is true that many others are capable of luxuriant growth on a completely vitamin-free medium. Do these latter organisms synthesize their own vitamins, or can they live and multiply without them? Because of the widespread occurrence of, and the widespread requirements for the B vitamins, and because of the established relationships between certain of these vitamins and enzyme systems of supposedly universal occurrence, it has been generally assumed that where microorganisms do not possess a dietary requirement for these vitamins, they possess the necessary mechanisms for synthesizing them. In support of this theory many workers have reported the synthesis of various B vitamins by certain microorganisms. Most of these reports have been of a qualitative nature, and many are concerned with the production of B vitamins by bacteria in the alimentary tract (1) (this bulletin, p. 37).

A quantitative study of the synthetic powers of bacteria as regards the B vitamins of necessity awaited the development of suitable assay methods. In 1941, Landy and Dicken (2) reported on the synthesis of biotin by microorganisms. Utilizing the assay method of Snell *et al.* (3) they demonstrated the synthesis of biotin by all the microorganisms tested, and found that the major portion of the biotin was found in the medium, rather than in the bacterial cells.

In this investigation five bacterial species were studied. They were *Aerobacter aerogenes, Serratia marcescens, Pseudomonas fluorescens, Proteus vulgaris,* and *Clostridium butylicum.* All but the last named are capable of growing on a completely vitamin-free medium. The medium employed was as follows:

Glucose	20 g.
Charcoal treated, vitamin-free casein hydrolysate	1 g.
Inorganic salts, solutions A and B (this bulletin, p. 13)	5 ml. each
Inorganic salts (Williams and Saunders) (4)	1 ml. each
Water to make	1 liter

For Clostridium butylicum one microgram of biotin per liter was added to the above medium.

The *Aerobacter, Serratia, Pseudomonas,* and *Proteus* were grown aerobically in shallow layers in twelve liter round-bottom flasks. The *Clostridium* and *Aerobacter* were grown anaerobically in an oat jar. All cultures were incubated for 24 hours at 33° C. A large inoculum (one-tenth the volume of the culture) of rapidly growing cells, grown in the medium described above, was used in order to insure heavy growth. By using a

large inoculum and a short growth period, it was hoped that a rapidly growing culture with few dead cells would be obtained for assay.

After incubation the bacterial cells were separated from the medium with the aid of a Sharples Super-Centrifuge. Typically, about three grams of moist cells were obtained from two liters of medium. The medium was sterilized and assayed for vitamin content without further treatment. The moist cells were suspended in sodium acetate–acetic acid buffer, pH 4.7, and an amount of the two enzyme preparations, takadiastase and papain, each equal to two per cent of the moist weight of the cells was added. This mixture was then allowed to autolyze under benzene for 24 hours at 37° C. After autolysis the mixture was steamed for thirty minutes to remove the benzene, and the solids removed by filtration. This procedure had been found to be the most satisfactory one for extraction of the B vitamins (5) (this bulletin, p. 15). In some cases, however, acid hydrolysis has been found to be somewhat more efficient in the extraction of biotin (6). Accordingly a portion of the bacterial cells was also autoclaved for an hour with six normal sulfuric acid. After neutralization and filtration these extracts were used for biotin determinations.

Microbiological assay methods described earlier in this bulletin (page 7) were used for the various vitamins. Satisfactory assays for inositol could not be obtained on the media due to interference by toxic substances.

The results of the assays are given in Tables I–VIII. It will be seen that every organism tested synthesizes a significant amount of each of the eight vitamins studied. It is interesting and significant that the amount of any given vitamin in the various bacterial cells assayed is relatively constant. Thus in the case of nicotinic acid, the variation is only from 200 to 250 micrograms per gram. Although not quite so marked with the other vitamins, the amount in the cells is in all cases relatively constant. No such constancy is found in the amount present in the medium. Apparently a certain amount of each of the vitamins is required for the activities of the cell, this amount being fairly constant for different species of bacteria. Any excess which may be synthesized is excreted into the medium. This explanation is given further weight by the fact that *Clostridium butylicum,* which requires biotin, hoards all of the biotin supplied to it within the cells, and leaves only a negligible amount free in the medium.

TABLE I

Thiamin Synthesis by Microorganisms

Organism	γ Thiamin Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	11	8.9	19.9
Aerobacter aerogenes (anaerobic)	15	11	26
Serratia marcescens	27	17	44
Pseudomonas fluorescens	26	48	74
Proteus vulgaris	21	<2.0	21–23
Clostridium butylicum	9.3	30	39.3

TABLE II

Riboflavin Synthesis by Microorganisms

Organism	γ Riboflavin Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	44	110	154
Aerobacter aerogenes (anaerobic)	54	25	79
Serratia marcescens	35	160	195
Pseudomonas fluorescens	67	310	377
Proteus vulgaris	57	38	95
Clostridium butylicum	55	180	235

TABLE III

Nicotinic Acid Synthesis by Microorganisms

Organism	γ Nicotinic Acid Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	240	390	630
Aerobacter aerogenes (anaerobic)	200	58	258
Serratia marcescens	240	230	470
Pseudomonas fluorescens	210	350	560
Proteus vulgaris	250	80	330
Clostridium butylicum	250	1680	1930

TABLE IV

Pantothenic Acid Synthesis by Microorganisms

Organism	γ P.A. Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	140	640	780
Aerobacter aerogenes (anaerobic)	340	410	750
Serratia marcescens	120	52	172
Pseudomonas fluorescens	91	220	311
Proteus vulgaris	100	<30	100–130
Clostridium butylicum	93	225	318

TABLE V

Pyridoxin Synthesis by Microorganisms

Organism	γ Pyridoxin Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	6.8	20	26.8
Aerobacter aerogenes (anaerobic)	18	<5	18–23
Serratia marcescens	11	23	34
Pseudomonas fluorescens	5.7	70	75.7
Proteus vulgaris	6.8	9.6	16.4
Clostridium butylicum	6.2	17	23.2

TABLE VI

Biotin Synthesis by Microorganisms

Organism	γ Biotin Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	3.9	44	47.9
Aerobacter aerogenes (anaerobic)	2.4	11	13.4
Serratia marcescens	4.1	30	34.1
Pseudomonas fluorescens	7.1	61	68.1
Proteus vulgaris	3.4	18	21.4
Clostridium butylicum	1.7	0.10	1.8

TABLE VII

Inositol Synthesis by Microorganisms

Organism	γ Inositol Per Gram Dry Cells		
	Cells	Medium	Total
Aerobacter areogenes (aerobic)	1400		
Aerobacter areogenes (anaerobic)	1600		
Serratia marcescens	1600		
Pseudomonas fluorescens	1700		
Proteus vulgaris	1000		
Clostridium butylicum	870		

TABLE VIII

Folic Acid Synthesis by Microorganisms

Organism	γ Folic Acid Per Gram Dry Cells*		
	Cells	Medium	Total
Aerobacter aerogenes (aerobic)	14	91	105
Aerobacter aerogenes (anaerobic)	5.0	20	25
Serratia marcescens	16.0	82	98
Pseudomonas fluorescens	8.8	66	74.8
Proteus vulgaris	21	21	42
Clostridium butylicum	2.8	16	18.8

*Micrograms of material of "potency" 40,000.

This regularity in the amount present in the cells, and irregularity in the amount found in the medium is illustrated by the "vitamin profiles" shown in Fig. 1, where the concentrations of the vitamins synthesized by the bacteria are compared with the concentrations of these vitamins in the normal rat carcass. The method of comparison used has been described by Taylor, Pollack, and Williams (7) (this bulletin, p. 41). The shaded portions represent the amounts in the cells while the unshaded portions represent the amounts excreted into the medium.

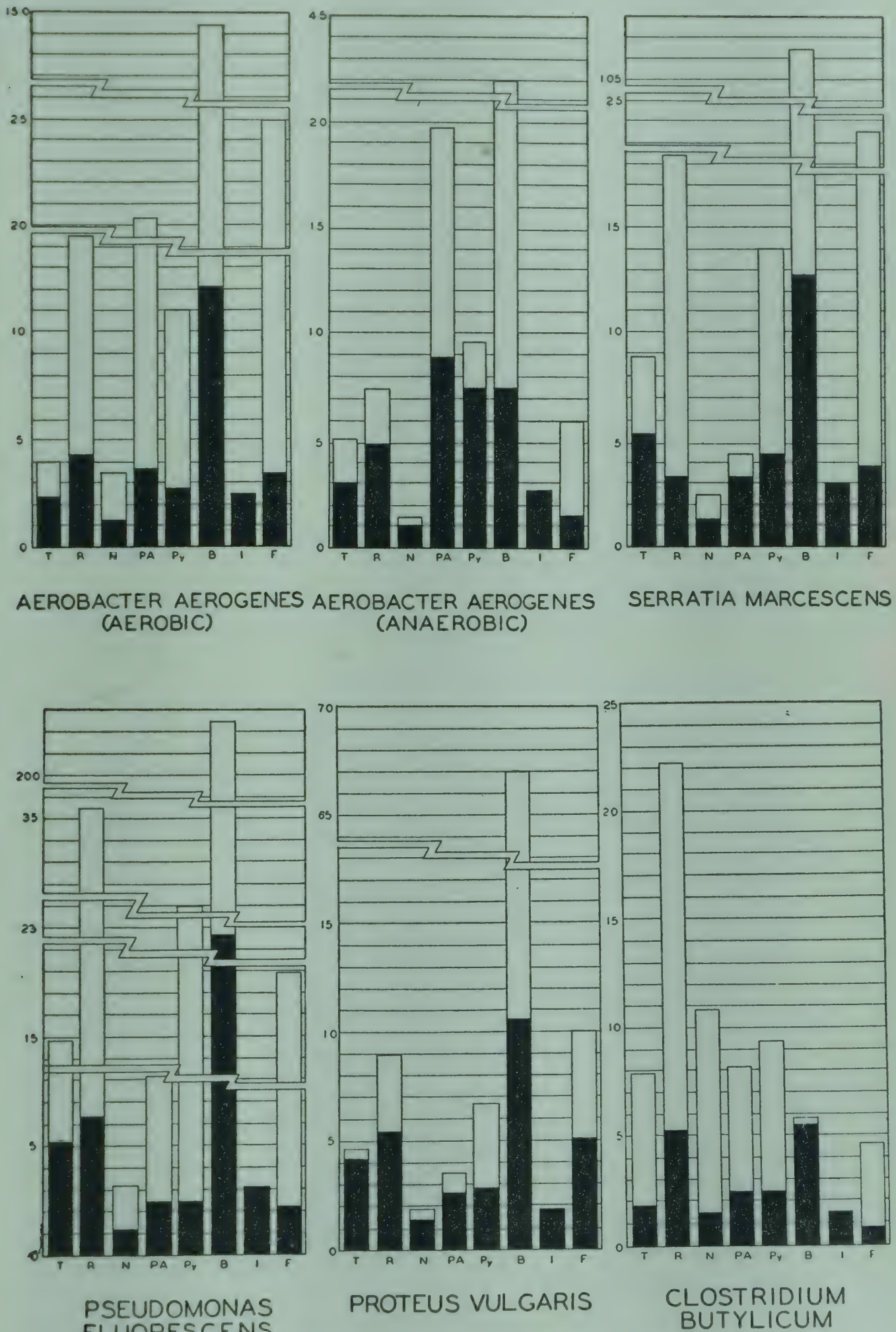

Fig. 1. "Vitamin Profiles." B Vitamins in Bacteria.

It will be seen that with the exception of nicotinic acid and biotin, the vitamins are on the average from two to five times as abundant in the bacterial cells as in the rat carcass. Biotin is much more abundant, while nicotinic acid is only slightly more abundant in the bacteria than in the rat carcass. The total production of vitamins (amount in cell plus amount in medium) varies widely among the species, but nevertheless shows some regularities. All species with the exception of *Clostridium butylicum*, produce enormous quantities of biotin. Riboflavin and folic acid are also produced in quantity by most of the species. Nicotinic acid production is uniformly low except in the case of *Clostridium butylicum*. As previously mentioned, satisfactory assays could not be obtained for inositol in the medium.

To check further the hypothesis that bacterial cells retain only a certain prescribed amount of a given vitamin, regardless of the amount of that vitamin present, cultures of *Aerobacter aerogenes* were grown in the same vitamin-free medium previously described, to which was added in one case one milligram per liter of riboflavin, in another five milligrams per liter of pantothenic acid. Bacterial cells grown both aerobically and anaerobically under the same conditions as previously described were then assayed, as was also the medium in which they had grown, for riboflavin and pantothenic acid. A comparison of the results obtained from bacteria grown in vitamin-free and riboflavin and pantothenic acid enriched media is given in Tables IX and X. It will be seen that although the amount of each of these vitamins present in the medium was increased tremendously, the amount within the cells remained essentially constant. Further, the amount liberated into the medium by the cells was not significantly increased.

TABLE IX

Comparison of Riboflavin Content of *Aerobacter Aerogenes* Cells Grown in Vitamin-Free and Riboflavin-Enriched Medium

Organism	Supplement	γ Riboflavin Per Gram Dry Cells		
		Cells	Medium	Total
Aerobacter aerogenes (aerobic)	none	44	110	154
Aerobacter aerogenes (anaerobic)	none	54	25	79
Aerobacter aerogenes (aerobic)	3520 γ riboflavin per gram of dry cells	55	3660	3715
Aerobacter aerogenes (anaerobic)	3130 γ riboflavin per gram of dry cells	69	3160	3229

A question that presents itself is whether the vitamins in the medium are secretory or excretory products of the living cell, or whether or not they appear in the medium merely as a result of autolysis of the dead cells. That autolysis was not responsible for the vitamin content of the media given in Tables I to X seems probable because of the short growth period (24 hrs.), and because of the regularity in the vitamin content of

TABLE X

Comparison of Pantothenic Acid Content of *Aerobacter Aerogenes* Cells Grown in Vitamin-Free and Pantothenic Acid-Enriched Medium

Organism	Supplement	γ Pantothenic Acid Per Gram Dry Cells		
		Cells	Medium	Total
Aerobacter aerogenes (aerobic)	none	140	640	780
Aerobacter aerogenes (anaerobic)	none	340	410	750
Aerobacter aerogenes (aerobic)	18,500 γ pantothenic acid per gram of dry cells	120	19,300	19,420
Aerobacter aerogenes (anaerobic)	15,900 γ pantothenic acid per gram of dry cells	250	11,400	11,650

the cells. That autolysis can play an important role was indicated by preliminary experiments, not reported here in detail, in which longer growth periods were used and erratically lower values were obtained for the vitamin content of the cells.

In the case of biotin it was possible to obtain direct experimental evidence as to whether autolysis was responsible for the presence of this vitamin in the culture medium. A 48-hour culture of *Proteus vulgaris* was grown from a small inoculum on the previously described vitamin-free medium. Samples of the culture were removed aseptically at intervals during the growth of the culture, the biotin content of the medium determined by microbiological assay, and the relative amount of growth determined turbidimetrically. The data thus obtained are given in Table XI and shown graphically in Fig. 2. Biotin liberation is seen to parallel growth

TABLE XI

Growth-Biotin Liberation Characteristics of *Proteus Vulgaris*

Time in Hours	Per Cent of Final Growth	Biotin Liberation	
		γ/Liter	Per Cent of Final Amount
4	7.1	.015	2.7
8	40.4	.28	51.0
12	57.2	.38	69.2
16	65.6	.43	79.2
24	73.8	.46	83.7
48	100	.55	100

and actually "leads" growth. If autolysis were responsible for liberation of the biotin, the appearance of the biotin in the medium should lag behind the growth of the organism. This method of study was not found to be applicable to the other vitamins because the methods of assay were not sufficiently sensitive to determine accurately the small amounts of the vitamin present in the medium during early stages of growth of the culture.

The question of vitamin interrelationships is one that has long attracted attention. It was hoped that some light might be shed on this problem by determining how the presence of a large amount of one vitamin would

affect the synthesis of the other B vitamins by bacteria. Accordingly cultures of *Aerobacter aerogenes* were grown on the previously described vitamin-free medium to which was added a large amount of one of the B vitamins. Such cultures were grown for each of the B vitamins except biotin (due to its unavailability in quantity in pure form). A control culture containing none of the vitamins was also grown. All eight cultures were grown under identical conditions. The vitamins excreted into

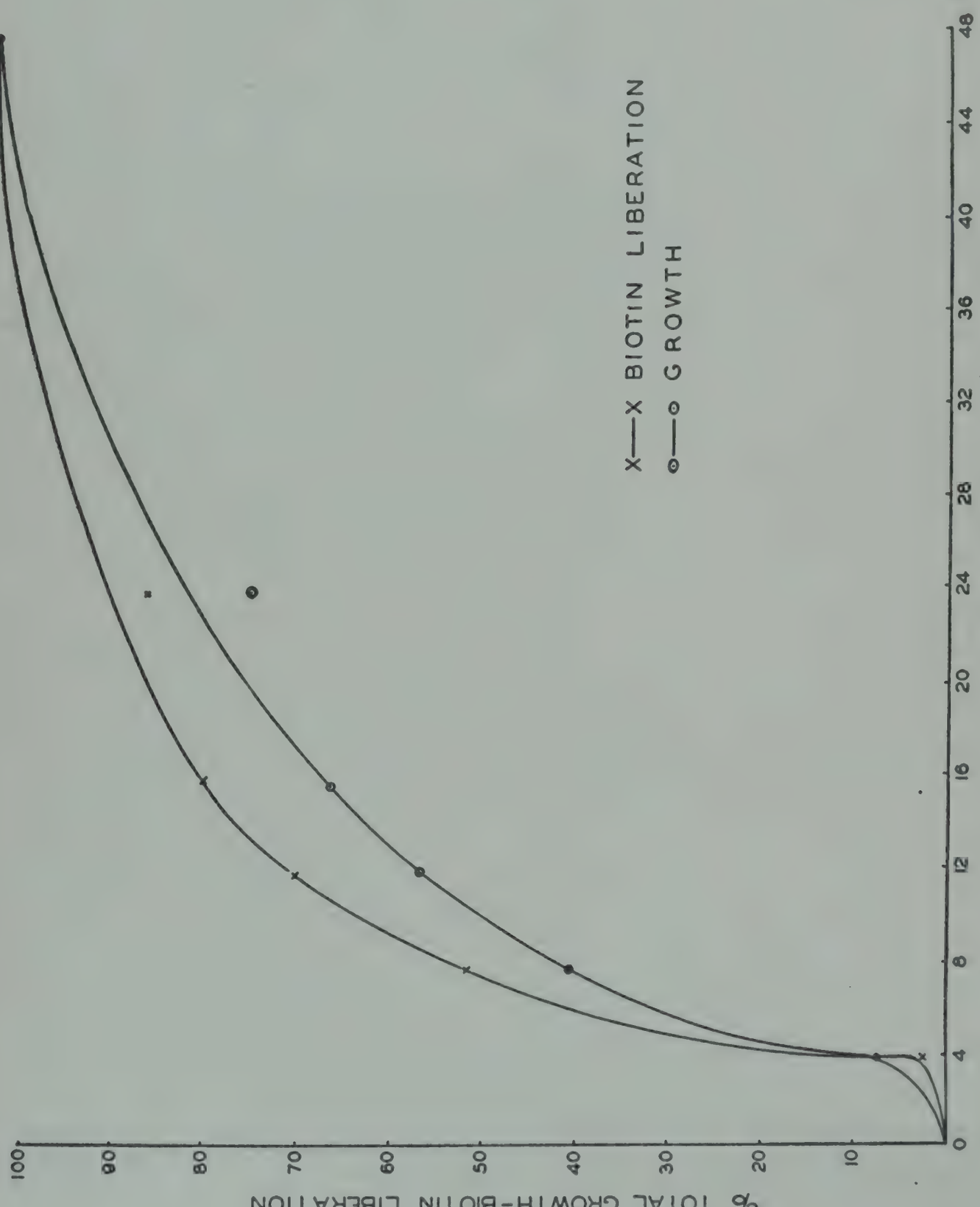

Fig. 2. Growth—Biotin Liberation Characteristics of *Proteus Vulgaris*.

the medium were then determined with the results shown in Table XII. It will be seen that the presence of a large amount of a single vitamin did not significantly affect the synthesis of any of the other vitamins.

TABLE XII

Effect of Supplementation with a Single B Vitamin on Production of other B Vitamins by *Aerobacter Aerogenes*

(Values in γ/Liter)

Medium Supplement	Relative Growth	Thiamin†	Ribo-flavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Folic* Acid
None	100	—	76	87	97	3.8	12	20
Thiamin (0.5 mg./l.)	117	140	110	120	150	7.1	12	25
Riboflavin (2.5 mg./l.)	89	2.1	2700	80	67	1.4	11	17
Nicotinic Acid (5 mg./l.)	106	—	85	4200	100	5.5	11	20
Pantothenic Acid (5 mg./l.)	93	1.5	79	76	3300	3.6	13	18
Pyridoxin (0.5 mg./l.)	104	—	86	86	85	270	13	25
Inositol (25 mg./l.)	100	1.7	94	65	130	3.7	13	18
Folic Acid (0.5 *mg./l.)	100	2.0	86	75	90	4.1	11	480

*Micrograms of material of "potency" 40,000.

†Satisfactory results could not be obtained with some samples due to the fact that the amount of thiamin was too low.

SUMMARY

1. All organisms tested synthesized significant quantities of all of the B vitamins.

2. The amount of a particular vitamin retained within the bacterial cell was relatively constant among the different species, while the amount found in the medium varied widely. This suggests that while synthetic powers vary, the amount required by the cells is relatively constant, and that this amount is retained by the cell and the remainder excreted into the medium.

3. The liberation of biotin into the medium during growth of a culture was shown to closely parallel growth, indicating that the vitamin was liberated by excretion rather than by autolysis of dead cells.

4. The average vitamin content of the bacterial cells was about two to five times that of the rat carcass, except for biotin which was 10–20 times as high as in the rat carcass and nicotinic acid which was only from 1 to 1.5 times as high.

5. The addition of large amounts of individual B vitamins to culture media did not affect the synthesis of other B vitamins by the bacteria growing in the media.

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THE B VITAMIN CONTENT OF MILK FROM ANIMALS OF DIFFERENT SPECIES

By

Roger J. Williams, Vernon H. Cheldelin, and Herschel K. Mitchell

The general biological importance of milk as a natural food would be difficult to exaggerate, and a comparison of the milks of different species from the standpoint of their content of B vitamins should throw important light on the subject of comparative nutrition and the relationship of B vitamins thereto.

In the present study, milk from six different species, including human, has been assayed for eight of the B vitamins (with minor exceptions), and in addition milks from different individual cows have been periodically assayed in order to learn something about the individual variability which may exist among animals.

PROCEDURE

The microbiological assay methods described earlier (this bulletin, p. 7) have been employed. Special "ultramicro" testing methods were employed for the assay of riboflavin (1), nicotinic acid (2), and pantothenic acid (3) in mouse milk (obtained by killing a lactating animal and expressing milk from the mammary tissues), since the samples collected ranged from 0.04 ml. to 0.08 ml. in volume. Even with the aid of these methods the quantities obtained were not sufficiently large to include assays for folic acid.

Extracts have been prepared by the general enzyme extraction procedure outlined previously (4) (this bulletin, p. 15). Certain additional precautions have been taken, however.

EFFECT OF ADDED ENZYMES

When tissues are digested with enzymes the amount of each vitamin contributed by the added enzymes is usually very small compared to the amount present in the tissues. Milk, on the other hand, is a poor source of some vitamins and the quantity present in the enzymes often assumes important proportions.

In Table I comparative data are listed showing the yield of B vitamins from whole milk and skim milk (commercial samples) when treated in different ways. In each case the increased yield due to enzyme digestion is compared to the calculated correction for the vitamins in the enzymes.

It is evident from Table I that enzyme digestion produces appreciable increases in the yields of biotin and inositol not only over autoclaving with steam, but also over hydrolysis with acid. Thiamin yields are also increased, as pointed out previously by others (5, 6). On the other hand,

TABLE I

Effect of Added Enzymes on Yields of B Vitamins from Milk
γ/ml.

	Thiamin		Nicotinic Acid		Pantothenic Acid		Pyridoxin		Biotin		Inositol		Folic Acid*	
	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim
Autolyzed 24 hrs.	0.02		0.31		2.6		0.023		0.031		20		0.02	
Autoclaved 30 min. at 15 lb pressure	0.15	0.11	0.33	0.67	2.5	3.4	0.071	0.021	0.033	0.080	45	84	0.01	0.11
Digested 24 hrs. with takadiastase and papain	0.31	0.16	1.1	1.7	2.9	5.4	0.082	0.083	0.053	0.19	300	260	0.06	0.16
Autoclaved, then digested 24 hrs. with takadiastase and papain									0.049					
Refluxed 6 hrs. with 6N HCl									0.030		75			
Autoclaved 45 min. with 6N H_2SO_4									0.034		51			
Increased yield over autoclaved samples due to enzymes	0.16	0.05	0.8	1.0	0.4	2.0	0.011	0.062	0.020	0.11	250	180	0.05	0.05
Calc. correction for vitamins in enzymes	0.07	0.07	1.0	1.0	0.5	0.5	0.040	0.040	0.004	0.004	110	110	0.08	0.08

*Micrograms of material of "potency" 40,000.

yields of the other vitamins in milk are not increased particularly by enzyme digestion. With some vitamins the "extra" yield is not sufficient to account for the vitamins added with the enzymes. The discrepancies are often greater for whole milk than for skim milk.

For each vitamin where these discrepancies occur, the calculated "correction" due to the added enzymes has been reduced to equal the observed increment obtained in the presence of the enzymes. No attempt is made here to explain the failure to extract all of each vitamin present, presumably from the enzymes. It seems probable, however, that no serious errors will be introduced by such a procedure.

COMPARISON OF MILK FROM DIFFERENT SPECIES

In Table II are listed the contents of eight B vitamins in milk from six species; namely human, mare, cow, goat, dog, and mouse. The species have been arranged in the order of decreasing time required to reach maturity. This arrangement will also furnish an order of increasing percentage of protein in the milk solids of each species (7).

It may be seen from Table II that in general the vitamin levels tend to increase progressively from the human to the mouse, in the same manner as the protein contents mentioned above. Thus the levels in the mouse are consistently highest and the dog second except in thiamin. Human and mare milk are generally the poorest sources, with mare milk often being poorer than human milk. The work of Houston and Kon (8) indicates that the vitamin levels of thiamin and riboflavin in rat milk (0.5–0.8 γ/ml. and 4–8 γ/ml. respectively) are in direct agreement with the trends outlined above. Thiamin in guinea pig milk is at a similar level (0.6 γ/ml.) but riboflavin is low, being about 0.9 γ/ml.

The levels of folic acid, riboflavin and nicotinic acid are noteworthy. Folic acid is seen to be present in much higher concentration in human and dog milk than in the milk of the herbivorous animals—mare, cow, and goat. Riboflavin is extremely low in mare milk, and is markedly lower in human milk than in cow milk. Nicotinic acid on the other hand is lower in cow milk than in human milk.

From the standpoint of vitamin nutrition, goat milk is significantly superior to cow milk only in nicotinic acid.

B VITAMINS IN INDIVIDUAL SAMPLES OF COW MILK

The levels of seven B vitamins have been determined in individual milk samples from sixteen cows in two local herds, taken at successive intervals of approximately four weeks. Folic acid was omitted because of the very low content of cows milk. The object of this study was to evaluate variations both among different cows and also for individual cows at different intervals.

TABLE II

B Vitamin Content of Milk from Animals of Different Species
γ/ml.

	Human White	Mare Thorough-bred	Cow Jersey, Guernsey	Goat Sanaan, Nubian Toggenberg	Dog English Bull	Mouse Albino, dba, C3H
Thiamin Content	0.09	0.06	0.44	0.39	0.05	5.7
No. samples	8	1	8	4	1	2
No. specimens sampled	4	1	8	4	1	2
Maximum and minimum values	0.23–<0.01		0.71–0.31	0.48–0.32		7.4–4.0
Riboflavin Content	0.38	0.02	0.95	1.1	3.0	10.4
No. samples	7	1	28	4	1	3
No. specimens	3	1	15	4	1	3
Maximum and minimum values	0.44–0.33		1.6–0.53	1.2–0.89		12–7.8
Nicotinic Acid Content	1.6	0.47	0.66	2.5	6.8	41
No. samples	9	1	26	4	1	3
No. specimens	5	1	14	4	1	3
Maximum and minimum values	2.2–1.2		1.2–0.19	3.2–2.0		50–34
Pantothenic Acid Content	1.6	2.9	2.9	2.4	4.9	23
No. samples	9	1	30	4	1	3
No. specimens	5	1	15	4	1	3
Maximum and minimum values	3.0–0.8		4.6–1.7	3.2–1.3		29–14
Pyridoxin Content	0.040	0.014	0.060	0.067	0.084	0.14
No. samples	9	1	23	4	1	3
No. specimens	5	1	14	4	1	3
Maximum and minimum values	0.086–0.019		0.11–0.026	0.13–0.036		0.25–0.082
Biotin Content	0.001	0.022	0.050	0.063	0.12	0.32
No. samples	8	1	30	4	1	3
No. specimens	4	1	15	4	1	3
Maximum and minimum values	0.005–<0.001		0.11–0.016	0.083–0.047		0.48–0.22
Inositol Content	330	180	180	210	440	1200
No. samples assayed	9	1	30	4	1	2
Total number of specimens	5	1	15	4	1	2
Maximum and minimum values	500–190		390–30	260–140		1500–930
Folic Acid Content*	0.45	<0.01	<0.05	<0.03	0.46	
No. samples	7	1	15	4	1	
No. specimens	3	1	15	4	1	
Maximum and minimum values*	0.50–0.33		<0.05–<0.01	<0.03–<0.01		

*In micrograms of material of 40,000 "potency."

Although differences are seen to exist (Table III) in the vitamin levels of different milk samples, they do not appear to follow definite patterns. This is especially true of Herd No. 1. In this group, samples which appear to be high in one or two vitamins are not necessarily above average in the others. Likewise there is little coherence in the assays of successive samples from each cow. Variations from the average are rather large except for pantothenic acid and thiamin.

Regularities are more apparent among the animals in Herd No. 2. Individual deviations from the herd averages are appreciably smaller, and successive samples from each cow often show agreement. This is especially true of pantothenic acid, riboflavin, and pyridoxin in the first two series of samples.

The differences in vitamin levels between the Jersey and Guernsey breeds appear to be negligible.

In a previous communication from this laboratory (9) it was reported that the concentrations of the various vitamins in animal tissues decreased in the following order: inositol, nicotinic acid, pantothenic acid, riboflavin, thiamin, pyridoxin, folic acid, and biotin. This order is observed for cow milk with the exception of nicotinic acid and folic acid, both of which are lower than might be expected on the basis of the above scheme. These two vitamins are appreciably richer in human milk (Table II).

It is interesting and perhaps significant to note that the samples observed in this study are somewhat lower in their riboflavin content than are the majority of samples tested by others. A number of these have been summarized recently by Munsell (10). Published thiamin values, on the other hand, are in very good agreement with those of the present study.

B VITAMINS IN INDIVIDUAL HUMAN MILK SAMPLES

The samples of human milk were obtained from five individuals in apparent good health and normal state of nutrition.*

Results of the assays are listed in Table IV. It may be noted that the vitamin levels are fairly uniform among Subjects Nos. 407, 410, and 44. Subjects No. 401 and 27 had been receiving daily vitamin supplements during pregnancy. This may account for the relatively higher levels of several vitamins in these samples.

Of special interest are the low levels of biotin and thiamin and the high content of folic acid in human milk. Similar thiamin values have been noted previously (11). The thiamin levels appear to increase slightly until about the 5th day of lactation, after which they diminish rapidly. Pyridoxin, inositol, and pantothenic acid also appear to diminish slightly during lactation. On the basis of this small number of assays, supplementation of mothers' diets with thiamin would appear advantageous.

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*We are indebted to these individuals, to Dr. T. J. McElhenney, Dr. Truman N. Morris, and to members of the Seton Hospital staff of Austin, Texas, for their coöperation in furnishing samples for assay.

TABLE III

B Vitamins in Milk from Individual Cows
Contents (γ/ml.)

Herd	Breed	Cow No.	Date	Milk Production, lb day	No. of days milked	Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol
1	Jersey	1	4–29–42	43	43	0.31	0.53	0.73	2.9	0.026	0.016	130
			5–19–42	38	63	0.39	1.1	0.90	3.4	0.075	0.050	180
		2	4–29–42	40	70	0.43	0.87	0.77	3.5	0.044	0.032	120
			5–19–42	40	90	0.22	1.0	0.83	2.5	0.060	0.047	
		3	4–29–42	35	52	0.46	0.86	0.95	4.0	0.026	0.035	200
			5–19–42	34	72	0.25	0.72	0.19	2.3	0.080	0.045	32
		4	4–29–42	34	77	0.35	1.6	1.1	4.1	0.059	0.031	160
			5–19–42	36	97	0.33	1.1	0.60	3.5	0.099	0.046	150
		5	4–29–42	30	112			0.87	4.5	0.038	0.039	180
			5–19–42	30	132	0.39	1.1	0.30	3.5	0.043	0.025	180
		6	4–29–42	28	119	0.38	1.2	0.79	4.6	0.040	0.055	280
		7	4–29–42	27	49	0.71	0.72		4.0	0.054	0.046	390
			5–19–42	27	69	0.25	1.4	0.67	2.8	0.094	0.027	120
		8	4–29–42	24	244	0.38	0.76	1.2	4.0	0.039	0.031	210
			5–19–42	24	264	0.30	1.1	0.80	3.4	0.083	0.056	230
		9	4–29–42	23	282	0.47		0.57	3.9	0.062	0.018	160
			5–19–42	24	302	0.33	0.96	0.75	3.1	0.075	0.037	190
		10	5–19–42	23		0.35	1.1	1.0	3.7	0.11	0.046	180
	Herd Average				4–29–42	0.44	0.93	0.87	3.9	0.043	0.034	200
					5–19–42	0.31	1.1	0.69	3.1	0.080	0.042	170

TABLE III—Continued

B Vitamins in Milk from Individual Cows
Contents (γ/ml.)

Herd	Breed	Cow No.	Date	Milk Production, lb day	No. of days milked	Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol
2	Guernsey	1	5–10–42	36	52	0.31	0.79	0.93	2.0	0.062	0.030	75
			6– 1–42	35	74	0.18	0.62	0.37	2.2	0.032	0.040	140
			6–30–42	32	103		1.1	0.72				220
		2	5–10–42	30	159	0.25	0.76	0.57	2.3	0.065	0.052	88
			6– 1–42	26	181	0.20	0.68	0.27	2.0	0.062	0.051	170
			6–30–42	26	212	0.28	1.0	0.99	3.4	0.055	0.11	260
		3	5–10–42	21	205		1.1				0.067	
			6– 1–42		227	0.15	1.1		3.0	0.049	0.062	160
			6–30–42	20	256	1.26	1.4	0.96	3.4	0.081	0.13	300
		4	5–10–42	21	120	0.25	1.2	0.78	2.6	0.040	0.077	200
			6– 1–42	20	142	0.17	1.2	0.20	1.7	0.042	0.080	210
			6–30–42	20	171	0.19	1.6	0.36	3.1	0.057	0.11	330
		5	5–10–42	20	154	0.19	0.75	0.57	1.9	0.062	0.040	220
			6– 1–42	17	176	0.71	0.74	0.70	2.8	0.041	0.069	190
		6	5–10–42	18	120	0.34	0.88		2.6	0.053	0.064	240
			6– 1–42	17	142	1.1	0.77	0.50	2.6	0.056	0.11	320
			6–30–42	20	171	0.37	1.3	0.43	3.4	0.065	0.14	250
		Herd Average			5–10–42	0.27	0.91	0.71	2.2	0.056	0.055	160
					6– 1–42	0.42	0.85	0.39	2.4	0.047	0.069	190
					6–30–42	0.28	1.3	0.69	3.3	0.063	0.12	270

TABLE IV

B Vitamins in Individual Human Milk Samples
(γ/ml.)

Subject No.	Period of Lactation Days	Thiamin	Riboflavin	Nicotinic Acid	Pantothenic Acid	Pyridoxin	Biotin	Inositol	Folic* Acid
407	3	0.08	0.41	1.5	1.0	0.035		380	0.50
407	5	0.11	0.33	1.4	2.0	0.041		420	0.44
407	8	<0.01	0.36	1.4	1.3	0.019	<0.002	220	0.45
410	3	0.08	0.37	1.5	1.4	0.037		500	0.46
410	5	0.10	0.44	1.2	1.8	0.032		270	0.47
410	8	0.05	0.41	1.4	1.4	0.025		300	0.48
44	13	0.07	----	2.2	0.77	0.020		330	----
401	10	0.23	0.36	2.1	2.1	0.086	0.005	190	0.33
27	20	0.40	0.15	1.9	3.0	0.070	0.018	330	0.44

*Micrograms of material of "potency" 40,000.

SUMMARY

1. Assays of samples of milk from animals of different species have been made for eight B vitamins. The species include human, mare, cow, goat, dog, and mouse. The vitamin levels were found generally to increase progressively from the human and mare to the mouse.

2. Individual samples of milk from 16 cows of two breeds have been assayed at intervals. Pantothenic acid, thiamin, and riboflavin levels exhibited the greatest degree of uniformity among the various samples tested. The other vitamin levels exhibited relatively little uniformity.

3. Individual human milk samples from five individuals have been assayed at intervals. A considerable degree of uniformity was found to exist for the various vitamins among most of the samples tested. Biotin and thiamin concentrations were found to be appreciably lower, and folic acid much higher, in human milk than in cow milk.

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THE B VITAMIN CONTENT OF FOODS

By

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The recognition of the importance of the B vitamins in nutrition and the widespread interest which has developed regarding their distribution in foodstuffs has made desirable the extension of our knowledge in this field. A number of studies of this nature have already been made, particularly with respect to those B vitamins which have been known for a longer time and for which reliable assay methods have been available.

The purpose of the present paper is to extend the scope of previous studies, both with regard to the number and variety of foodstuffs usually tested and also with regard to the newer B vitamins, about which our present knowledge is meager.

SELECTION AND SAMPLING OF FOODS

All foods except milk and flesh foods were purchased in nearby markets in as fresh condition as possible. Milk was obtained from a number of individual cows at the time of milking. Meats were, with few exceptions, obtained from a single wholesale butcher so that it was possible in most cases to be assured of freshness of the samples. Fish were bought frozen as "Birds-Eye" products.

Perishable foods were kept at 0–5° until they were prepared for assay. These preparations were made within a few hours after procurement of the samples.

PROCEDURE

The microbiological assay methods referred to on page 7 of this bulletin have been employed throughout for each of the eight vitamins herein discussed. The general enzyme digestion procedure described earlier (1) (this bulletin, p. 32) has been followed for preparation of all food extracts.

RESULTS

In Table I are given the results of the analyses of the edible portions of 53 representative foods, for each of the following vitamins: thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxin, biotin, inositol, and folic acid. They represent cereal products, flesh foods, vegetables, fruits, and a few miscellaneous items.

COMPARISON AND DISCUSSION OF RESULTS

A high degree of correlation is known to exist among several of the B vitamins with respect to the amounts present in various animal tissues (2). Although the values observed for foods from various sources

have not been compared mathematically, it is of interest to observe the relative range of abundance of each vitamin in the foods studied. Thus, the concentration of thiamin in pork muscle, the richest source, is 150 times as great as in fresh peaches, the poorest source found. Similar ranges of concentration of the other vitamins between their richest and poorest sources are: riboflavin 210, nicotinic acid 1,800, pantothenic acid 190, pyridoxin 230, biotin 330, inositol 440, and folic acid 210, all measured on the dry weight basis. The relative ranges of concentration for these foods are thus seen to be nearly alike for all the vitamins studied with the exception of nicotinic acid. The ranges for all the B vitamins are in marked contrast to the fat soluble vitamins, where the richest tissues may (due to storage) contain several million times as much of a given vitamin as do poorer tissues. The degree to which certain tissues store B vitamins is not yet known, but storage is generally considered to take place to a small extent in this group.

The method used in sampling meats should be considered carefully whenever fat meats are to be assayed. The rather common practice among a number of workers of trimming away all visible fat from meat samples before preparing them for assay is probably not warranted in cases where kitchen practice includes the adhering fat as edible tissue, even though the results so obtained are more likely to be reproducible. In this manner the apparent vitamin contents of ham, bacon or fat mutton may be markedly exaggerated, especially when they are reported on a dry weight basis.

THIAMIN

Numerous studies have been made on the thiamin content of many materials. Various methods of assay have been used. Reliable assay methods in use at the present time are the rat curative, yeast fermentation and thiochrome methods. Each of these was found to give comparable results when tested on a group of cereal samples (3), and these methods have been standardized by the Research Corporation Committee on Assay Methods.

Recently a survey of the vitamin content of foods has been made by Lane, Johnson, and Williams (4) using the thiochrome assay method. Nordgren and Andrews (5) and Conner and Straub (6) have also assayed a large number of cereals by this method.

A comparison of the results of Table I using the yeast growth method (this bulletin, p. 11) with those obtained by the above workers reveals good agreement among most of the values. Only in the case of roasted peanuts, chocolate and molasses, all of which have been subjected to cooking, is there striking disagreement. Fresh peanuts assayed 7 γ per gram by the thiochrome method as contrasted with 14 γ by the yeast growth method. Roasting decreased the thiochrome value (same sample) to about 2.8 γ per gram. Thiochrome assays show essentially no thiamin in molasses. It appears evident that the yeast growth method cannot safely be applied

to materials which have been subjected to treatments which may destroy thiamin.

Assays of fresh pork by the present method give somewhat lower values than those reported by Lane and coworkers and those of Waisman and Elvehjem (7). We are inclined to regard these lower values as being due to the individual samples tested, since the values reported here for bacon and ham agree reasonably well with those of the above workers.

The thiamin contribution of various foods to the average American diet has been determined by Lane, Johnson, and Williams. The rather low content of thiamin (0.8 mg.) in an ordinary 2,500 calorie diet is reflected by the fact that few foods other than pork are rich sources of thiamin. On a fresh weight basis whole wheat compares favorably but when dry weights are considered, pork is approximately six times as rich a source as wheat. Other muscle meats are only fair sources; beef round contains only about five to ten per cent as much thiamin as pork. Organ tissues are somewhat richer, with heart and brain being fairly good sources of this vitamin. Other moderate to good sources are liver, wheat germ, cabbage, peas, cauliflower, beet greens, carrots, tomatoes and grapefruit. Processed cereals, milk, and fresh peaches are relatively poor sources.

RIBOFLAVIN

A large number of papers dealing with the riboflavin content of certain natural products has appeared in the literature. Most of these, however, have been primarily concerned with assay of selected materials of relatively high potency. Recently Waisman and Elvehjem (7) have investigated the riboflavin content of a number of meats, using the Snell and Strong microbiological assay method and Munsell has assayed a number of other foods by the rat growth method (8).

The riboflavin values in Table I represent an extension of the number of foodstuffs previously assayed for this vitamin. Insofar as duplication of materials occur, the present values are generally in good agreement with those of Munsell (8), Waisman and Elvehjem (7), Conner and Straub (9), and Hodson and Norris (10).

Riboflavin, although present in all the foods studied, has few rich sources. On a dry weight basis, liver, heart, milk, eggs, and leafy green vegetables are the best sources. Wheat products, non-leafy vegetables and most fresh fruits are relatively low in riboflavin content.

NICOTINIC ACID

The nicotinic acid content of foods, especially of meats, has received considerable study in recent months (7, 11). Values obtained by other workers are generally in good agreement with those presented in Table I in the case of fresh vegetables and flesh foods.

Teply, Strong, and Elvehjem (11) found somewhat greater amounts of nicotinic acid in cereals and certain fruits than have been found in the present study. However, their assays were made upon extracts which had

been prepared by acid hydrolysis. The higher values obtained for cereals are therefore to be expected, as pointed out previously (this bulletin, p. 23). The reason for the discrepancies among fruit assays is not known.

Flesh foods and whole wheat products are in general the best sources of nicotinic acid. Fresh fruits and vegetables are relatively much lower in their nicotinic acid content. Liver, chicken breast muscle, and halibut, as well as mushrooms, are unusually rich sources, whereas milk and eggs are extremely poor sources of the vitamin.

PANTOTHENIC ACID

The present study marks the first quantitative estimate of the distribution of pantothenic acid among a large variety of foods, using the microbiological technique. Previous estimates using chick assays have been reported by Jukes (12) and Waisman and Elvehjem (7). In general there is good agreement between the two methods, although materials of low potency tend to give lower values by chick assay than by microbiological assay. Microbiological assays of meats by Waisman and Elvehjem (7) are also in good agreement with the present values.

Pantothenic acid is distributed abundantly and rather evenly in a number of foods of different type. Thus, while it is present in much smaller quantities in meats than is nicotinic acid (liver is an exception) it is present in comparable or greater amounts in common foods such as milk, eggs, carrots, cauliflower, peas, beans, and several fresh fruits. In addition to these foods, others such as animal organs, mushrooms, and tomatoes are good sources of this vitamin.

Only a few of the foods studied are very poor sources of pantothenic acid. These include such items as raisins, apples, peaches, cheese, and processed cereals. In the present study we have found veal muscle to be a relatively poor source of this vitamin, but the tissues used appear to be exceptional since the data of Waisman and Elvehjem give considerably higher values both by chick and microbiological assay.

PYRIDOXIN

Pyridoxin assays by the yeast growth method are much lower than vitamin B_6 values obtained by other methods (7) but appear from our experimental findings to be specific for pyridoxin itself (13, 14).

From Table I it is apparent that fresh vegetables are rich sources of pyridoxin, often better than meats. The best vegetables are cabbage, lettuce, carrots, potatoes, sweet potatoes, and turnips. Bananas, tomatoes, and oranges, together with wheat germ, are also good sources. Milk, eggs, and processed cereals are poor sources.

Among the meat samples tested, considerable variation is found among different samples, especially among cured meats. Fresh muscle meats are fair sources of pyridoxin.

The fact that liver tissue, not only of beef but other species as well (2), is only a fair source of pyridoxin indicates that storage of this vitamin does not take place in this organ. This is in marked contrast to most of

the other vitamins. Vitamin B_6 (as determined by animal assays) (7) has also been found to be relatively low in liver, indicating a similar failure of the liver to store this material.

BIOTIN

The biotin contents of certain foods have been reported by Lampen, Bahler, and Peterson (15) using *Cl. butylicum* as the test organism. The agreement between the data of these workers and those presented in Table I is only fair, with the present results being somewhat higher in a number of instances. The Wisconsin workers reported their assays for extracts prepared by autoclaving with water and by hydrolysis with 2N or 4N H_2SO_4. In several instances the values obtained were higher for the water extracts than for the acid hydrolysates, indicating that some destruction had taken place during the acid treatment. Destruction by acid has also been noted by others. In view of this fact it is probable that some of the values obtained by acid hydrolysis are too low, even in cases where all of the "bound" biotin is released from tissues by this means.

Biotin appears to be distributed somewhat equally among many of the foods investigated. On a dry weight basis, milk, egg, most fresh vegetables, and several fruits are good sources of biotin, whereas wheat and corn products are consistently poor. In confirmation of the findings of Lampen, Bahler, and Peterson, lean beef and pork are not very good sources of the vitamin, but the organs are appreciably richer; liver was found to be the richest of all foods studied.

INOSITOL

Inositol is the one B vitamin among those studied which is present in foods in far greater absolute amount than any of the others. Its richest source is oranges where it makes up 1.6% of the dry weight of the fruit (16,000 γ/gram).

Like pyridoxin, and unlike most of the other B vitamins, inositol is only moderately abundant in muscle meats or other foods of primarily protein nature. Heart is an important exception, being one of the richest sources found.

Fruits and vegetables, which in general are relatively low in protein content as compared to meats, are often excellent sources of inositol; many of them contain more than 4,000 γ per gram of dry tissue. Brain, with inositol present in phospholipid combination (16) is a very rich source, and wheat germ and whole wheat are also good sources. Milk and eggs contain moderate amounts, while processed cereals, lean beef, and fish are very low in their inositol content.

As was observed with pyridoxin, inositol does not appear to be stored in the liver in amounts appreciably higher than in muscle (2). Liver from beef as well as from other species contains somewhat more inositol than skeletal muscle, but much less than brain or heart tissue.

FOLIC ACID

Folic acid, as its name implies, is especially abundant in green leafy vegetables, although these substances are not the only good sources. Among the other vegetables, carrots, mushrooms, blackeyed peas, and cauliflower, are all rich in folic acid, as are several fruits, notably watermelons, cantaloupes, tomatoes, and oranges. Wheat germ is a good source but other cereals are moderate to poor.

Oysters and liver are good sources of folic acid, but muscle meats are at best only fair sources; beef round and pork loin contain only one-sixth to one-tenth as much folic acid as spinach on a dry weight basis. Among the muscle meats studied, chicken appears to be the best source of folic acid. The general distribution of folic acid in foods is seen here to bear some resemblance to that of pyridoxin and inositol.

Cow's milk, as pointed out previously (this bulletin, p. 99) is unique in its extremely low content of folic acid.

TABLE I

B Vitamin Contents of Foods

Cereal Products
γ/gm.

Material	Whole Wheat	Wheat germ	Bread Whole Wheat	Bread White Unenriched	Flour White Unenriched
Per cent Dry Weight	90	89	65	65	89
Thiamin content, fresh	5.0	18	2.3	0.78	0.73
Thiamin content, dry	5.6	20	3.5	1.2	0.82
No. samples assayed	1	1	1	1	1
Total No. specimens sampled	1(lb)	1(lb)	1	1	1(lb)
Max. and min. values					
Riboflavin Content, fresh	1.6	4.8	1.1	—	0.36
Riboflavin Content, dry	1.8	5.3	1.7	—	0.40
No. samples	1	1	2	—	2
Total specimens	1(lb)	1(lb)	2	—	2(lb)
Max. and min. values	—	—	1.1–1.03	—	0.39–0.22
Nicotinic Acid Content, fresh	41	22	28	6.0	6.1
Nicotinic Acid Content, dry	46	24	42	9.2	6.9
No. samples	1	1	1	2	3
Total specimens	1(lb)	1(lb)	1	2	3(lb)
Max. and min. values	—	—	1	6.2–5.8	7.9–4.3
Panto. Acid Content, fresh	12	20	5.7	4.6	3.5
Panto. Acid Content, dry	13	22	8.8	6.9	3.9
No. samples	1	1	2	1	2
Total specimens	1(lb)	1(lb)	2	2	2(lb)
Max. and min. values	—	—	6.1–5.3	—	3.7–3.2
Pyridoxin Content, fresh*	2.1	6.0	0.38	0.039	0.20
Pyridoxin Content, dry	2.2	6.7	0.58	0.060	0.22
No. samples	1	1	2	1	2
Total specimens	1(lb)	1(lb)	2	1	2(lb)
Max. and min. values	—	—	0.41–0.38	—	0.23–0.16
Biotin Content, fresh	0.052	—	0.019	0.011	0.007
Biotin Content, dry	0.058	—	0.029	0.016	0.008
No. samples	1	—	1	1	2
Total specimens	1(lb)	—	1	1	2(lb)
Max. and min. values	—	—	—	—	0.007–0.007
Inositol Content, fresh	1700	6900	670	510	740
Inositol Content, dry	1890	7700	1030	780	830
No. samples	1	1	2	1	2
Total specimens	1(lb)	1(lb)	2	1	2(lb)
Max. and min. values	—	—	810–500	—	850–630
Folic Acid Content, fresh†	1.9	11	0.69	0.34	0.67
Folic Acid Content, dry	2.0	12	1.1	0.53	0.75
No. samples	1	1	2	1	1
Total specimens	1(lb)	1(lb)	2	1	1(lb)
Max. and min. values	—	—	0.86–0.52	—	—

*This is not equivalent to "vitamin B_6" as determined by other methods.
†Micrograms of material having a "potency" of 40,000.

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Material	Cereal Products: Corn Meal White	Cereal Products: Hominy Grits	Cereal Products: Rice Krispies	Dairy Products: Milk Whole	Dairy Products: Cheese
Percent Dry Weight	88	89	96	12.1	62–79
Thiamin Content, fresh	----	----	0.81	0.44	0.26
Thiamin Content, dry	----	----	0.85	3.6	0.39
No. Samples Assayed	----	----	1	8	1
Total No. Spec. Sampled	----	----	1 (lb)	8	1
Max. and Min. Values	----	----	----	0.71–0.31	----
Riboflavin Content, Fresh	0.63	0.55	0.47	0.95	5.9
Riboflavin Content, Dry	0.72	0.62	0.49	7.8	8.3
No. Samples	1	1	1	28	5
Total Specimens	1 (lb)	1 (lb)	1 (lb)	15	5
Max. and Min. Values	----	----	----	1.6–0.53	7.0–5.1
Nico. Acid Content, Fresh	7.5	2.0	6.9	0.66	0.2
Nico. Acid Content, Dry	8.5	2.2	7.2	5.5	0.3
No. Samples	2	1	1	26	3
Total Specimens	2 (lb)	1 (lb)	1 (lb)	14	3
Max. and Min. Values	8.5–6.5	----	----	1.2–0.19	0.4–0.1
Panto. Acid Content, Fresh	3.1	1.0	3.4	2.9	2.3
Panto. Acid Content, Dry	3.5	1.1	3.5	24	3.4
No. Samples	2	1	1	30	1
Total Specimens	2 (lb)	1 (lb)	1 (lb)	15	1
Max. and Min. Values	3.2–2.9	----	----	4.6–1.7	----
Pyridoxin Content, Fresh	0.54	0.05	0.48	0.060	0.66
Pyridoxin Content, Dry	0.61	0.06	0.50	0.50	0.98
No. Samples	1	1	1	23	1
Total Specimens	1 (lb)	1 (lb)	1 (lb)	14	1
Max. and Min. Values	----	----	----	0.11–0.026	----
Biotin Content, Fresh	0.058	0.006	0.013	0.050	0.036
Biotin Content, Dry	0.066	0.007	0.014	0.41	0.054
No. Samples	1	1	1	30	1
Total Specimens	1 (lb)	1 (lb)	1 (lb)	15	1
Max. and Min. Values	----	----	----	0.11–0.016	----
Inositol Content, Fresh	450	32	190	180	250
Inositol Content, Dry	510	36	200	1400	380
No. Samples	1	1	1	30	1
Total Specimens	1 (lb)	1 (lb)	1 (lb)	15	1
Max. and Min. Values	----	----	----	390–30	----
Folic Acid Content, Fresh	0.20	0.10	0.16	<0.05	0.30
Folic Acid Content, Dry	0.23	0.11	0.17	<0.4	0.45
No. Samples	1	1	1	15	1
Total Specimens	1 (lb)	1 (lb)	1 (lb)	15	1
Max. and Min. Values	----	----	----	<0.05–<0.01	----

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Material	Dairy Products		Beef		
	Butter	Eggs	Round	Liver	Heart
Percent Dry Weight	85	26.0	29.5	35.8	23.4
Thiamin Content, Fresh	----	1.4	0.63	2.6	4.4
Thiamin Content, Dry	----	5.4	2.1	7.3	19
No. Samples Assayed	----	2	1	1	2
Total No. Spec. Sampled	----	12	1	1	3
Max. and Min. Values	----	1.6–1.2	----	----	4.8–4.0
Riboflavin Content, Fresh	0.12	4.4	2.2	28	8.8
Riboflavin Content, Dry	0.14	17	7.5	78	38
No. Samples	1	8	1	2	2
Total Specimens	1	90	4	5	3
Max. and Min. Values	----	6.4–2.2	----	28–27	8.8–8.8
Nico. Acid Content, Fresh	----	0.72	46	120	84
Nico. Acid Content, Dry	----	2.8	160	340	360
No. samples	----	4	2	1	2
Total Specimens	----	42	5	1	3
Max. and Min. Values	----	1.0–0.67	46–45	----	87–81
Panto. Acid Content, Fresh	----	14	4.9	76	20
Panto. Acid Content, Dry	----	54	17	210	85
No. Samples	----	2	2	3	3
Total Specimens	----	12	5	6	4
Max. and Min. Values	----	14–14	5.1–4.7	92–55	23–18
Pyridoxin Content, Fresh	----	0.22	0.77	1.7	1.2
Pyridoxin Content, Dry	----	0.85	2.6	4.7	5.1
No. Samples	----	2	1	1	1
Total Specimens	----	12	4	1	2
Max. and Min. Values	----	0.28–0.16	----	----	1.3–1.1
Biotin Content, Fresh	----	0.090	0.026	0.96	0.081
Biotin Content, Dry	----	0.35	0.077	2.6	0.34
No. Samples	----	2	3	3	3
Total Specimens	----	12	6	6	4
Max. and Min. Values	----	0.123–0.056	0.030–0.022	1.13–0.87	0.13–0.049
Inositol Content, Fresh	----	330	115	510	2600
Inositol Content, Dry	----	1300	390	1400	11000
No. Samples	----	2	1	1	2
Total Specimens	----	12	1	1	3
Max. and Min. Values	----	470–190	----	----	2900–2200
Folic Acid Content, Fresh	----	0.86	1.0	3.8	1.1
Folic Acid Content, Dry	----	3.3	3.4	11	4.7
No. Samples	----	2	1	2	2
Total Specimens	----	12	4	5	3
Max. and Min. Values	----	0.95–0.76	----	4.3–3.2	1.6–0.56

TABLE I—Continued

B Vitamin Contents of Foods

γ/gm.

Material	Beef Brain	Pork Loin I	Pork Loin II*	Pork Loin III*	Pork Bacon I
Percent Dry Weight	22.0	39	27	30	78
Thiamin Content, Fresh	2.3	7.9	7.7	6.3	3.7
Thiamin Content, Dry	10.4	20	28	21	4.7
No. Samples Assayed	1	1	1	1	1
Total No. Spec. Sampled	1	4	3	3	2
Max. and Min. Values					
Riboflavin Content, Fresh	1.4	2.8	1.5	2.0	1.3
Riboflavin Content, Dry	6.3	7.2	5.5	6.7	1.7
No. Samples	1	2	1	1	1
Total Specimens	1	5	3	3	2
Max. and Min. Values	-----	3.2–2.3	------	------	------
Nico. Acid Content, Fresh	35	38	90	51	13
Nico. Acid Content, Dry	160	98	330	170	17
No. Samples	1	1	1	1	1
Total Specimens	1	4	3	3	2
Max. and Min. Values					
Panto. Acid Content, Fresh	18	11	4.7	5.8	2.8
Panto. Acid Content, Dry	81	28	17	19	3.6
No. Samples	1	2	1	1	1
Total Specimens	1	5	3	3	2
Max. and Min. Values	-----	14–7.1	------	------	------
Pyridoxin Content, Fresh	-----	0.86	1.00	2.7	0.29
Pyridoxin Content, Dry	-----	2.2	3.7	9.1	0.37
No. Samples	-----	1	1	1	1
Total Specimens	-----	4	3	3	2
Max. and Min. Values					
Biotin Content, Fresh	0.074	0.046	0.020	0.038	0.074
Biotin Content, Dry	0.33	0.12	0.074	0.13	0.095
No. Samples	1	2	1	1	1
Total Specimens	1	5	3	3	2
Max. and Min. Values	-----	0.055–0.036	------	------	------
Inositol Content, Fresh	2000	450	360	440	430
Inositol Content, Dry	9100	1200	1300	1500	550
No. Samples	1	1	1	1	1
Total Specimens	1	4	3	3	2
Max. and Min. Values					
Folic Acid Content, Fresh	0.52	0.65	0.84	1.4	0.60
Folic Acid Content, Dry	2.4	1.7	3.1	4.7	0.77
No. Samples	1	1	1	1	1
Total Specimens	1	4	3	3	2
Max. and Min. Values					

*All visible fat removed before preparation of extracts.

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Material	Pork			Veal	
	Bacon II*	Ham I	Ham II*	Chop I	Chop II*
Percent Dry Weight	49.7	52	33.5	32.0	27.9
Thiamin Content, Fresh	7.6	9.8	8.7	0.65	1.7
Thiamin Content, Dry	15	19	26	2.0	6.1
No. Samples Assayed	1	1	1	1	1
Total No. Spec. Sampled	12	2	1	6	3
Max. and Min. Values					
Riboflavin Content, Fresh	3.1	2.5	2.5	2.2	1.4
Riboflavin Content, Dry	6.2	4.8	7.5	6.9	5.0
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					
Nico. Acid Content, Fresh	42	33	31	72	70
Nico. Acid Content, Dry	85	63	93	230	250
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					
Panto. Acid Content, Fresh	9.8	3.4	6.6	1.1	2.6
Panto. Acid Content, Dry	20	6.5	20	3.4	9.3
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					
Pyridoxin Content, Fresh	1.0	0.19	1.7	0.56	1.3
Pyridoxin Content, Dry	2.0	0.37	5.1	1.8	4.7
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					
Biotin Content, Fresh	0.079	0.060	0.040	0.020	0.014
Biotin Content, Dry	0.16	0.12	0.12	0.064	0.050
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	4	3
Max. and Min. Values					
Inositol Content, Fresh	640	310	580	320	350
Inositol Content, Dry	1300	600	1700	1000	1300
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					
Folic Acid Content, Fresh	1.6	0.58	1.2	0.92	1.7
Folic Acid Content, Dry	3.2	1.1	3.6	2.9	6.1
No. Samples	1	1	1	1	1
Total Specimens	12	2	1	6	3
Max. and Min. Values					

*All visible fat removed before preparation of extracts.

TABLE I—Continued

B Vitamin Contents of Foods

γ/gm.

Material	Lamb Leg*	Mutton Shoulder	Chicken Leg	Chicken Breast	Sea Foods Halibut
Percent Dry Weight	30.5	42.3	22.3	25.1	27.0
Thiamin Content, Fresh	2.8	0.51	0.90	0.74	0.73
Thiamin Content, Dry	9.2	1.2	4.0	3.0	2.7
No. Samples Assayed	1	1	2	2	2
Total No. Spec. Sampled	1	4	4	2	2
Max. and Min. Values	-----	-----	1.01–0.78	0.77–0.71	0.75–0.71
Riboflavin Content, Fresh	2.4	2.5	2.6	1.2	0.67
Riboflavin Content, Dry	7.9	5.9	12	4.8	2.5
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	2.7–2.4	1.3–1.0	0.89–0.43
Nico. Acid Content, Fresh	75	40	38	91	110
Nico. Acid Content, Dry	250	95	170	360	410
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	43–32	105–79	140–75
Panto. Acid Content, Fresh	6.0	4.3	6.2	5.3	1.5
Panto. Acid Content, Dry	20	10.2	28	21	5.6
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	7.7–5.2	5.5–5.1	1.9–1.1
Pyridoxin Content, Fresh	0.81	0.18	0.25	1.3	1.1
Pyridoxin Content, Dry	2.7	0.43	1.1	5.2	4.1
No. Samples	1	1	1	1	2
Total Specimens	1	4	2	1	2
Max. and Min. Values	-----	-----	-----	-----	1.3–0.84
Biotin Content, Fresh	0.021	0.027	0.098	0.054	0.080
Biotin Content, Dry	0.069	0.064	0.44	0.21	0.30
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	0.11–0.083	0.058–0.050	0.094–0.065
Inositol Content, Fresh	580	500	470	480	170
Inositol Content, Dry	1900	1200	2000	1900	610
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	560–370	670–280	190–140
Folic Acid Content, Fresh	1.1	0.77	1.2	1.5	0.71
Folic Acid Content, Dry	3.6	1.8	5.4	6.0	2.7
No. Samples	1	1	2	2	2
Total Specimens	1	4	4	2	2
Max. and Min. Values	-----	-----	1.5–0.95	2.0–0.98	0.99–0.43

*All visible fat removed before preparation of extracts.

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

	Sea Foods		Vegetables		
Material	Salmon	Oyster	Beans Dried Lima	Beets	Beet Greens
Percent Dry Weight	28.1	19.7	92.4	13.4	10.3
Thiamin Content, Fresh	1.3	1.8	5.3	0.26	1.4
Thiamin Content, Dry	4.6	9.1	5.8	1.9	14
No. Samples Assayed	1	1	1	1	1
Total No. Spec. Sampled	1	4	1(lb)	3	5
Max. and Min. Values					
Riboflavin Content, Fresh	1.4	2.2	1.3	0.60	2.1
Riboflavin Content, Dry	5.0	11	1.4	4.5	20
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Nico. Acid Content, Fresh	64	12	9.8	6.5	6.0
Nico. Acid Content, Dry	230	61	11	49	58
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Panto. Acid Content, Fresh	6.6	4.9	8.3	1.1	1.4
Panto. Acid Content, Dry	24	25	9.0	8.2	14
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Pyridoxin Content, Fresh	0.33	0.33	5.5	0.13	0.37
Pyridoxin Content, Dry	1.2	1.7	6.0	0.97	3.6
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Biotin Content, Fresh	0.053	0.087	0.098	0.003	0.027
Biotin Content, Dry	0.19	0.44	0.11	0.022	0.26
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Inositol Content, Fresh	170	440	1700	210	210
Inositol Content, Dry	600	2200	1800	1600	2000
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					
Folic Acid Content, Fresh	0.87	2.4	3.3	0.45	2.1
Folic Acid Content, Dry	3.1	12	3.6	3.4	20
No. Samples	1	1	1	1	1
Total Specimens	1	4	1(lb)	3	5
Max. and Min. Values					

TABLE I—Continued

B Vitamin Contents of Foods

γ/gm.

Material	Vegetables Cabbage	Carrots	Cauliflower	Lettuce	Mushrooms
Percent Dry Weight	8.3	11.8	11.7	5.2	12.5
Thiamin Content, Fresh	1.4	0.49	1.9	0.39	1.1
Thiamin Content, Dry	17	4.2	16	7.5	8.8
No. Samples Assayed	1	3	2	1	1
Total No. Spec. Sampled	1	12	2	1	3
Max. and Min. Values	-----	0.71–0.35	2.4–1.4	-----	-----
Riboflavin Content, Fresh	0.57	0.50	1.3	0.27	3.3
Riboflavin Content, Dry	6.9	4.2	11	5.2	26
No. Samples	1	4	2	2	1
Total Specimens	1	31	2	2	3
Max. and Min. Values	-----	0.64–0.42	1.3–1.2	0.33–0.20	-----
Nico. Acid Content, Fresh	2.1	2.6	5.7	2.5	69
Nico. Acid Content, Dry	25	22	50	48	550
No. Samples	1	4	2	1	1
Total Specimens	1	18	2	1	3
Max. and Min. Values	-----	2.8–2.3	6.5–4.8	-----	-----
Panto. Acid Content, Fresh	1.8	2.5	9.2	1.1	17
Panto. Acid Content, Dry	22	21	80	21	140
No. Samples	1	4	2	1	1
Total Specimens	1	18	2	1	3
Max. and Min. Values	-----	2.7–1.9	10.3–8.1	-----	-----
Pyridoxin Content, Fresh	1.2	1.2	0.20	0.71	0.45
Pyridoxin Content, Dry	14	10.2	1.7	14	3.6
No. Samples	1	2	1	1	1
Total Specimens	1	10	1	1	3
Max. and Min. Values	-----	1.2–1.1	-----	-----	-----
Biotin Content, Fresh	0.024	0.025	0.17	0.031	0.16
Biotin Content, Dry	0.29	0.21	1.5	0.60	1.3
No. Samples	1	4	2	1	1
Total Specimens	1	18	2	1	3
Max. and Min. Values	-----	0.032–0.017	0.19–0.14	-----	-----
Inositol Content, Fresh	950	480	950	550	170
Inositol Content, Dry	11000	4100	8100	10000	1400
No. Samples	1	3	2	1	1
Total Specimens	1	9	2	1	3
Max. and Min. Values	-----	670–260	1100–800	-----	-----
Folic Acid Content, Fresh	0.65	0.97	1.4	0.38	0.98
Folic Acid Content, Dry	7.8	8.2	12	7.3	7.8
No. Samples	1	4	2	1	1
Total Specimens	1	18	2	1	3
Max. and Min. Values	-----	1.3–0.75	1.6–1.1	-----	-----

TABLE I—Continued

B Vitamin Contents of Foods

γ/gm.

Vegetables

Material	Okra	Onions Dry	Peas		
			Dried English	Black-eyed	Green English
Percent Dry Weight	10.2	12.5	92	95	25.0
Thiamin Content, Fresh	1.2	0.36	5.9	8.1	3.5
Thiamin Content, Dry	12	2.9	6.4	8.8	14
No. Samples Assayed	1	1	1	1	2
Total No. Spec. Sampled	10	5	1(lb)	1(lb)	2(lb)
Max. and Min. Values	—	—	—	—	5.4–1.6
Riboflavin Content, Fresh	1.04	0.24	1.5	1.4	1.5
Riboflavin Content, Dry	10.2	1.9	1.6	1.5	6.0
No. Samples	1	2	3	1	1
Total Specimens	10	14	3(lb)	1(lb)	1(lb)
Max. and Min. Values	—	0.24–0.23	1.7–1.1	—	—
Nico. Acid Content, Fresh	7.1	0.8	28	13	18
Nico. Acid Content, Dry	70	6.4	30	14	72
No. Samples	1	1	2	1	2
Total Specimens	10	5	2(lb)	1(lb)	2(lb)
Max. and Min. Values	—	—	28–27	—	21–15
Panto. Acid Content, Fresh	2.1	1.3	—	10.4	3.8
Panto. Acid Content, Dry	21	10.4	—	11	15
No. Samples	1	1	—	1	2
Total Specimens	10	5	—	1(lb)	2(lb)
Max. and Min. Values	—	—	—	—	4.5–3.1
Pyridoxin Content, Fresh	0.75	0.63	3.0	1.9	0.79
Pyridoxin Content, Dry	7.3	5.0	3.3	2.1	3.2
No. Samples	1	1	1	1	1
Total Specimens	10	5	1(lb)	1(lb)	1(lb)
Max. and Min. Values					
Biotin Content, Fresh	0.055	0.035	0.18	0.21	0.094
Biotin Content, Dry	0.54	0.28	0.20	0.23	0.38
No. Samples	1	1	1	1	2
Total Specimens	10	5	1(lb)	1(lb)	2(lb)
Max. and Min. Values	—	—	—	—	1.2–0.67
Inositol Content, Fresh	530	880	3300	2400	1620
Inositol Content, Dry	5200	7000	3600	2500	6500
No. Samples	1	1	1	1	2
Total Specimens	10	5	1(lb)	1(lb)	2(lb)
Max. and Min. Values	—	—	—	—	1740–1490
Folic Acid Content, Fresh	0.53	0.13	—	7.4	1.3
Folic Acid Content, Dry	5.2	1.1	—	8.0	5.2
No. Samples	1	1	—	1	2
Total Specimens	10	5	—	1(lb)	2(lb)
Max. and Min. Values	—	—	—	—	1.3–1.2

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Vegetables

Material	Potatoes: Irish	Potatoes: Sweet (Yams)	Spinach	Turnips
Percent Dry Weight	22.2	29.2	10.3	8.0
Thiamin Content, Fresh	1.7	1.2	0.56	0.26
Thiamin Content, Dry	7.6	4.1	5.4	3.3
No. Samples Assayed	1	2	1	1
Total Specimens	1	4	1(lb)	7
Max. and Min. Values	—	1.3–1.2	—	—
Riboflavin Content, Fresh	0.29	0.40	2.3	0.65
Riboflavin Content, Dry	1.3	1.4	22	8.1
No. Samples	3	3	2	1
Total Specimens	22	20	2(lb)	7
Max. and Min. Values	0.36–0.23	0.45–0.36	2.3–2.2	—
Nico. Acid Content, Fresh	4.3	5.5	5.1	6.9
Nico. Acid Content, Dry	19	19	50	86
No. Samples	3	3	1	1
Total Specimens	22	20	1(lb)	7
Max. and Min. Values	5.3–3.8	6.3–5.0	—	—
Panto. Acid Content, Fresh	3.2	9.4	1.8	0.37
Panto. Acid Content, Dry	41	32	17	4.6
No. Samples	1	2	2	1
Total Specimens	1	4	2(lb)	7
Max. and Min. Values	—	9.8–9.0	2.0–1.6	—
Pyridoxin Content, Fresh	2.2	3.2	0.83	1.1
Pyridoxin Content, Dry	9.9	11	8.1	14
No. Samples	1	2	1	1
Total Specimens	1	4	1(lb)	7
Max. and Min. Values	—	3.5–2.8	—	—
Biotin Content, Fresh	0.006	0.043	0.069	0.021
Biotin Content, Dry	0.027	0.14	0.67	0.26
No. Samples	1	2	2	1
Total Specimens	1	4	2(lb)	7
Max. and Min. Values	—	0.049–0.037	0.78–0.59	—
Inositol Content, Fresh	290	660	270	460
Inositol Content, Dry	1300	2900	2600	5800
No. Samples	1	2	1	1
Total Specimens	1	4	1(lb)	7
Max. and Min. Values	—	840–480	—	—
Folic Acid Content, Fresh	1.4	0.67	2.4	0.26
Folic Acid Content, Dry	6.3	2.3	23	3.3
No. Samples	1	2	2	1
Total Specimens	1	4	2(lb)	7
Max. and Min. Values	—	0.84–0.50	3.0–1.7	—

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Fruits

Material	Apples	Bananas	Cantaloupe	Grapefruit	Oranges
Percent Dry Weight	14	24	9.7	11	13
Thiamin Content, Fresh	0.96	1.6	0.75	1.5	0.92
Thiamin Content, Dry	6.6	6.6	7.7	13	7.2
No. Samples Assayed	1	1	1	1	1
Total No. Spec. Sampled	5	6	3	4	6
Riboflavin Content, Fresh	0.18	0.56	0.26	0.30	0.47
Riboflavin Content, Dry	1.2	2.3	2.7	2.7	3.7
No. Samples	7	3	1	1	2
Total Specimens	57	10	3	4	12
Max. and Min. Values	0.23–0.13	0.78–0.43	-----	-----	0.51–0.42
Nico. Acid Content, Fresh	0.81	5.8	10.1	2.1	3.0
Nico. Acid Content, Dry	5.6	24	104	19	23
No. Samples	4	1	1	1	1
Total Specimens	38	6	3	4	6
Max. and Min. Values	0.98–0.61	-----	-----	-----	-----
Panto. Acid Content, Fresh	0.60	1.8	2.3	2.9	3.4
Panto. Acid Content, Dry	4.1	7.4	24	26	27
No. Samples	1	1	1	1	1
Total Specimens	6	6	3	4	6
Pyridoxin Content, Fresh	0.26	3.2	0.36	0.09	0.80
Pyridoxin Content, Dry	1.8	13	3.7	0.81	6.3
No. Samples	1	1	1	1	1
Total Specimens	5	6	3	4	6
Biotin Content, Fresh	0.009	0.044	0.031	0.030	0.019
Biotin Content, Dry	0.062	0.18	0.32	0.27	0.15
No. Samples	1	1	1	1	1
Total Specimens	5	6	3	4	6
Inositol Content, Fresh	240	340	1200	1500	2100
Inositol Content, Dry	1600	1400	12000	13000	16000
No. Samples	1	1	1	1	1
Total Specimens	6	6	3	4	6
Folic Acid Content, Fresh	0.08	0.95	1.3	0.55	0.83
Folic Acid Content, Dry	0.55	3.9	13	4.9	6.5
No. Samples	1	1	1	1	1
Total Specimens	5	6	3	4	6

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Fruits

Material	Peaches (Frozen)	Raisins	Strawberries	Tomatoes	Watermelon
Percent Dry Weight	26.0	76	10.0	6.0	10.0
Thiamin Content, Fresh	0.05	2.3	0.35	0.60	0.56
Thiamin Content, Dry	0.19	3.0	3.5	10	5.6
No. Samples Assayed	1	1	1	2	1
Total No. Spec. Sampled	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	0.73–0.46	-----
Riboflavin Content, Fresh	0.17	0.29	0.34	0.37	0.69
Riboflavin Content, Dry	0.65	0.38	3.4	6.2	6.9
No. Samples	2	3	1	3	1
Total Specimens	6	3 (lb)	12 oz.	11	1
Max. and Min. Values	0.18–0.15	0.36–0.20	-----	0.62–0.24	-----
Nico. Acid Content, Fresh	3.3	2.9	2.2	4.7	2.4
Nico. Acid Content, Dry	13	3.8	22	78	24
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	5.6–3.8	-----
Panto. Acid Content, Fresh	1.7	0.90	2.6	3.7	3.1
Panto. Acid Content, Dry	6.5	1.2	26	62	31
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	4.0–3.4	-----
Pyridoxin Content, Fresh	0.16	0.94	0.44	0.60	0.33
Pyridoxin Content, Dry	0.62	1.2	4.4	10.0	3.3
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	0.68–0.51	-----
Biotin Content, Fresh	0.017	0.031	0.040	0.040	0.036
Biotin Content, Dry	0.062	0.041	0.40	0.67	0.36
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	0.049–0.031	-----
Inositol Content, Fresh	960	1200	600	460	640
Inositol Content, Dry	3700	1600	6000	7700	6400
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	530–390	-----
Folic Acid Content, Fresh	0.17	0.28	0.23	0.75	1.5
Folic Acid Content, Dry	0.65	0.37	2.3	13	15
No. Samples	1	1	1	2	1
Total Specimens	3	1 (lb)	12 oz.	6	1
Max. and Min. Values	-----	-----	-----	1.1–0.40	-----

TABLE I—Continued

B Vitamin Contents of Foods
γ/gm.

Miscellaneous

Material	Chocolate	Molasses	Roasted Peanuts	Sugar	"Royal Jelly" (honey bee)
Percent Dry Weight	99	76	98	100	32
Thiamin Content, Fresh	6.4	5.9	14	——	6.6
Thiamin Content, Dry	6.4	7.8	14	——	21
No. Samples Assayed	1	1	1	——	3
Total No. Spec. Sampled	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	7.4–5.6
Riboflavin Content, Fresh	2.4	0.62	1.05	——	8.2
Riboflavin Content, Dry	2.4	0.82	1.1	——	26
No. Samples	3	1	2	——	3
Total Specimens	3(lb)	1	2(lb)	——	3
Max. and Min. Values	2.6–2.1	——	1.1–0.98	——	10–6.6
Nico. Acid Content, Fresh	11	39	86	——	59
Nico. Acid Content, Dry	11	51	88	——	190
No. Samples	1	1	1	——	3
Total Specimens	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	48–73
Panto. Acid Content, Fresh	1.9	2.6	25	——	89
Panto. Acid Content, Dry	1.9	3.4	25	——	290
No. Samples	1	1	1	——	3
Total Specimens	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	110–65
Pyridoxin Content, Fresh	0.23	2.7	3.0	——	2.4
Pyridoxin Content, Dry	0.23	3.6	3.1	——	7.7
No. Samples	1	1	1	——	3
Total Specimens	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	2.5–2.2
Biotin Content, Fresh	0.32	0.091	0.34	<0.004	1.7
Biotin Content, Dry	0.32	0.12	0.35	——	5.4
No. Samples	1	1	1	1	3
Total Specimens	1(lb)	1	1(lb)	1	3
Max. and Min. Values	——	——	——	——	1.8–1.6
Inositol Content, Fresh	850	1500	1800	——	100
Inositol Content, Dry	860	2000	1800	——	310
No. Samples	1	1	1	——	3
Total Specimens	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	150–78
Folic Acid Content, Fresh	0.99	0.095	2.8	——	0.20
Folic Acid Content, Dry	1.00	0.13	2.9	——	0.62
No. Samples	1	1	1	——	3
Total Specimens	1(lb)	1	1(lb)	——	3
Max. and Min. Values	——	——	——	——	0.22–0.16

SUMMARY

Fifty-three representative foods have been assayed for their content of thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxin, biotin, inositol, and folic acid. The richest and poorest sources of each are tabulated below.

Vitamin	Best Sources	Poor Sources
Thiamin	Pork, green vegetables, tomatoes, grapefruit, wheat germ.	Processed cereals, milk, beef muscle, fresh peaches.
Riboflavin	Organ tissues, milk, eggs, leafy green vegetables.	Cereal products, non-leafy vegetables, fresh fruits.
Nicotinic Acid	Mushrooms, flesh foods, liver, whole wheat.	Processed cereals, milk, eggs, fresh fruits.
Pantothenic Acid	Liver and other organs, many fresh vegetables and fruits.	Processed cereals, raisins, apples, peaches, cheese.
Pyridoxin	Fresh vegetables, several fruits.	Processed cereals, milk, eggs.
Biotin	Liver and other organs, fresh vegetables and fruits, poultry and dairy products, sea foods.	Cereals, apples, raisins.
Inositol	Fresh fruits, many vegetables, heart, brain, wheat germ.	Processed cereals, lean beef, fish.
Folic Acid	Green vegetables, several fruits, wheat germ.	Milk, lean beef, and pork.

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INTESTINAL BACTERIAL SYNTHESIS AS A SOURCE OF B VITAMINS FOR THE RAT

By

Herschel K. Mitchell and Edith R. Isbell

Recent experiments in an attempt to determine the possible effect of folic acid (1) on rat growth led us to suspect that the substance was being synthesized in considerable amounts by the intestinal flora of the animals. Since assay methods for several B vitamins were available, a general exploratory study of their production by rat intestinal flora *in vivo* was undertaken.

An examination of the recent literature revealed a number of investigations on the production of B vitamins by rat intestinal bacteria. Leong (2) carried out a balance study of thiamin on rats. Bacterial synthesis was not considered significant since fecal material contained a relatively small quantity of the vitamin. The destruction of thiamin in the animal body at high intake levels was demonstrated. Abdel-Salaam and Leong (3) demonstrated the synthesis of thiamin by a mixed flora taken from a rat cecum and grown *in vitro*. The vitamin was found in the bacterial cells and not in the surrounding medium. The production of riboflavin by different types of intestinal bacteria was studied by Tange (4). It was found that feces from animals fed on lactose or dextrinized corn starch had a greater effect in curing riboflavin deficiency in rats than did feces from animals fed on glucose, sucrose or corn starch as a carbohydrate source. In similar experiments, but extended to riboflavin, pyridoxin and the "filtrate factor," Morgan, Cook, and Davidson (5) fed to rats, rat cecal bacteria from animals having different dietary carbohydrates. Lactose was found to favor bacterial production of riboflavin and pyridoxin. Corn starch favored "filtrate factor" production, while sucrose had little influence on vitamin production. Shourie and Swaminathan (6) found that rats on low nicotinic acid dietary intakes (5–22 γ per day) excreted as high as 60 γ per day, while rats on high intakes (1,250 γ per day) excreted about 85 γ per day.

The suggestion has been made that variations in the kind of carbohydrate in the diet may affect the production of vitamins by intestinal flora by changing the type of bacteria or by reducing or increasing the number of bacteria. Extensive studies have been made on the effect of diet on the species of intestinal bacteria. The flora generally found has been divided into two main groups, coliform organisms and acidophylic organisms. Each of these may include a number of specific bacteria in varied proportions. Certain yeasts and molds are also found in relatively small numbers. Various workers have found that lactose favors the growth of acidophylic organisms (Mitchell (7), Ruickshank (8), Schieblich (9), and Kessel (10)). This type of intestinal organism was also favored by

cranberries (Weinstein and Weiss (11)), and by dried apples (Sullivan and Manville (12)), in the diet. The decrease in acidophylic flora as influenced by increased fat in the diet was demonstrated by Forti (13).

Coliform flora are produced by high protein diets, as shown by work of Porter, Weinstein, and Rettger (14). Experiments by Eppright, Valley, and Smith (15) indicated that a diet low in inorganic salts favored coliform flora.

Various substances, as sulfanilylguandine (Marshall, Bratton, White, and Litchfield (16)), alkyl resorcinol (Ratcliffe (17)), and reduced iron (Handke (18)), have been used to modify types of intestinal flora.

A summary of the results from the literature cited as well as our own preliminary experiments suggested that the bacteria in the intestines of a rat synthesize considerable quantities of B vitamins. It appeared probable that at least part of these were absorbed from the cecum or colon and might thus partially or completely satisfy the requirements of a rat for particular vitamins. Still further it appeared probable that different types of bacteria present in the intestines, as influenced by the diet, may contribute vitamins to the animal in amounts depending on the types of bacteria. Experiments were designed which, it was hoped, would contribute toward answering the following questions:

1. Do intestinal bacteria act as a source for each of the B vitamins for the rat; if so, about what proportion of each do they contribute?
2. Is the type or the number of intestinal bacteria, as modified by the diet of the rats an important factor in vitamin contribution by the flora?

EXPERIMENTAL

Diets to be used in the experimental work were chosen first on the basis of the type of bacterial flora they induce and second on the basis of similarity of constituents.

Diet A	Ground lean beef	100%
Diet B	Ground lean beef	75%
	Lactose	25%

According to the work of Porter and Rettger (19), Diet A should induce coliform flora, while Diet B should give largely acidophylic organisms. It was hoped that the predominance of lean beef in both diets would serve to minimize digestive and metabolic differences, with respect to the vitamins, between groups of rats on Diets A and B.

A litter of eight white rats, weaned at twenty-one days, was kept on a diet of Purina dog chow for a period of thirty-two days. After this time the rats, including males and females, varied in weight from 96 to 132 grams. The animals were then separated into two groups of four and placed on Diets A and B respectively for a period of thirty days. Two rats of each group were then transferred to cages similar to those of Selye, Bassett, and Nielson (20) in order that their excrement could be

collected for assay. These cages were designed to prevent washing of fecal material by the urine of the animals.

Experiment I. Two animals on Diet A were each fed 25 grams of ground meat once daily. The two on Diet B received 15 grams of ground meat and 4.5 grams of lactose daily. Urine was collected in bottles containing 100 milligrams of phenol. The collecting funnels were washed down and replaced with sterile funnels twice a day. The urine collected was extracted twice with ether to remove the phenol and then sterilized by autoclaving 15 minutes at 15 pounds pressure. Fecal material was also collected twice daily and sterilized to prevent further bacterial action. All samples obtained over a period of 105 hours from animals on the same diet were combined and mixed thoroughly. The four rats were killed 16 hours from the last feeding by giving a light ether anaesthesia, followed by an intraperitoneal injection of nembutal. Cecal contents from rats on the same diet were combined and mixed thoroughly. Aliquots were taken for bacteriological examination and the remainder sterilized. The intestinal tracts were removed from the freshly killed rats, freed from intestinal contents, and rinsed in cold saline solution (0.87%). The tracts were cut into five sections; the stomach, the upper half of the small intestine, the lower half of the small intestine, the cecum, and the colon. The intestinal sections from rats on the same diets were combined, ground in a microgrinder and extracted immediately with boiling water. The whole operation required less than one hour from the time the animals were killed. Samples of the diets, feces, and cecal contents were prepared for vitamin assays by enzymatic hydrolysis using a mixture of takadiastase and papain (21). Urine samples were tested without preliminary treatment. The hot water extracts of the intestinal wall samples were also not hydrolyzed since it was desired that only unbound vitamins be determined. The microbiological assay methods described earlier (this bulletin, p. 7) have been used throughout.

Bacteriological examinations of cecal contents and feces were carried out using the technique of Porter, Weinstein, and Rettger (14).

Experiment II. The second experiment involving the other four rats of the original group was essentially a repetition of the first, but some changes were made. The amount of food was changed to 27 grams a day for Diet A and 13 grams of meat and 3.9 grams of lactose daily for Diet B. The rats had been maintained on their original respective meat and meat-lactose diets during the interval between Experiments I and II. The same sample of beef was used throughout each experiment but a different sample was used in the two experiments. The determination of unbound vitamins in the intestinal walls was not repeated. Vitamins in the medium surrounding the bacteria of the cecum were determined in this experiment. Samples were prepared by suspending cecal contents in water and centrifuging until no bacteria remained in suspension. The extracts were not enzymatically hydrolyzed. Other samples were prepared as in Experiment I.

In Experiment I all four rats were males, while in Experiment II rats on Diet A were females and those on Diet B were one male and one female.

RESULTS

Some general information on the results of the balance studies is summarized in Table I.

TABLE I

	Experiment I		Experiment II	
	Diet A	Diet B	Diet A	Diet B
Ml. urine daily	16	13	19	19
Gms. feces daily	0.16	0.48	0.18	0.44
Gms. meat daily	25	15	27	13
Gms. lactose daily	0	4.5	0	3.9
Ave. gain daily	1.7	0	1.0	— 1.4
Gms. cecal contents	3.3	7.4	2.4	5.9

Data from vitamin assays calculated on a daily basis and as balance ratios of $\frac{\text{output}}{\text{intake}}$ are given in Table II. Values for seven of the vitamins are given in *micrograms* (γ) per day. Folic acid is given as micrograms of material of "potency" 40,000 per day. The results obtained in Experiments I and II are indicated after each vitamin in horizontal columns, while values from Diets A and B are included under separate vertical columns.

Table III summarizes results of assays on cecal contents enzymatically hydrolyzed and on cold water extracts. Values are given as *micrograms per gram* of moist weight of cecal material, except folic acid, which is given as micrograms of material of "potency" 40,000 per gm. In Experiment I the dry weight of cecal material from Diet A was 37% and from Diet B 44% of the moist weight.

Table IV summarizes assays on the intestinal wall sections from Experiment I. Vitamin values are given as *micrograms per gram* of moist tissue, except folic acid, which is given as micrograms of material of "potency" 40,000 per gram.

Bacteriological examinations on cecal material gave the results indicated in Table V. These values represent rough approximations only, due to the limitations of the methods of determination of the bacterial types (19).

TABLE II

Vitamin Balance for Rats on Different Diets

Vitamin	Expt.	Intake γ/day		Urine γ/day		Feces γ/day		Output γ/day		Output / Intake	
		Diet A	Diet B	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B
Thiamin	I	31	18	2.9	2.1	1.1	5.2	4.0	7.3	0.13	0.40
	II	36	17	0.94	0.84	1.4	3.7	2.3	4.5	0.064	0.26
Riboflavin	I	120	72	8.4	7.2	2.2	6.1	11	13	0.088	0.19
	II	40	20	3.7	1.7	3.9	7.4	7.6	9.1	0.19	0.45
Nicotinic Acid	I	860	520	110	59	15	43	130	100	0.15	0.2
	II	1700	810	130	63	24	58	150	120	0.09	0.15
Pantothenic Acid	I	120	69	100	83	3.5	26	100	110	0.92	1.7
	II	120	60	89	50	5.1	18	94	68	0.76	1.1
Pyridoxin	I	3.0	1.8	7.6	2.9	0.7	0.77	8.3	3.7	2.8	2.0
	II	3.5	1.7	11	1.5	0.71	1.2	12	2.7	3.3	1.6
Biotin	I	0.38	0.23	0.41	0.30	0.14	0.16	0.55	0.46	1.5	2.0
	II	0.68	0.33	0.19	0.26	0.10	0.11	0.29	0.37	0.43	1.1
Inositol	I	13000	7500	100	190	110	170	210	370	0.016	0.048
	II	19000	9100	170	170	67	110	240	280	0.013	0.031
Folic Acid	I	11	6.5	0.10	0.26	0.61	1.9	0.71	2.2	0.066	0.33
	II	8.1	3.9	0.21	0.16	1.4	2.7	1.6	2.9	0.18	0.73

TABLE III

B Vitamin in Cecal Contents of Rats

Vitamin	Experiment I: Cecum contents (Enzyme Hydrolyzed) γ/gm. Diet A	Experiment I: Cecum contents (Enzyme Hydrolyzed) γ/gm. Diet B	Experiment II: Cecum contents (Enzyme Hydrolyzed) γ/gm. Diet A	Experiment II: Cecum contents (Enzyme Hydrolyzed) γ/gm. Diet B	Experiment II: Cecum contents (water extract) γ/gm. Diet A	Experiment II: Cecum contents (water extract) γ/gm. Diet B
Thiamin	4.5	5.6	7.4	6.1	0.8	0.3
Riboflavin	4	6	11	8.2	0.9	0.8
Nicotinic Acid	30	42	40	47	3.7	3.8
Pantothenic Acid	11	22	23	24	1.7	8.6
Pyridoxin	1.0	0.5	-----	1.3	0.96	0.5
Biotin	0.47	0.46	0.25	0.22	0.19	0.21
Inositol	400	290	600	400	<2.0	<2.0
Folic Acid	0.91	0.86	3.9	6.4	0.68	1.0

TABLE IV

B Vitamins in Intestinal Walls of Rats

Experiment I

Vitamin	Stomach γ/gm. Diet A	Stomach γ/gm. Diet B	Upper Small Intestine γ/gm. Diet A	Upper Small Intestine γ/gm. Diet B	Lower Small Intestine γ/gm. Diet A	Lower Small Intestine γ/gm. Diet B	Cecum γ/gm. Diet A	Cecum γ/gm. Diet B	Colon γ/gm. Diet A	Colon γ/gm. Diet B
Folic Acid	0.32	0.43	0.55	0.22	0.13	0.32	0.4	0.57	0.55	0.41
Biotin	0.049	0.043	0.11	0.049	0.060	0.054	0.12	0.10	0.07	0.046
Pantothenic Acid	2.5	3.4	2.0	6.0	3.3	6.0	2.0	11	2.5	6.5

TABLE V

	Experiment I		Experiment II	
	Diet A Per cent	Diet B Per cent	Diet A Per cent	Diet B Per cent
Coliform	90	20	75	20
Acidophylic	10	80	25	80

DISCUSSION

Numerous experiments have been described in the literature showing that intestinal bacteria synthesize various B vitamins. The fact that these substances may be kept within the bacterial cells and may thus not be available to the animal has been indicated by work of Abdel-Salaam and Leong (3). Further evidence on this subject is given in Table III, where a comparison is presented between the total vitamin content of the cecal bacteria and the vitamin content of the medium surrounding the bacteria. Certain vitamins (inositol, nicotinic acid, riboflavin, and thiamin) are found in considerable quantities in the bacteria but diffuse into the surrounding medium to a relatively small extent. Pantothenic and folic acids appear to diffuse to a somewhat larger extent, while biotin and pyridoxin apparently move freely from the cells into the surrounding medium. Obviously the vitamins in the medium are those which may become available to the animal and these values are the ones which should be considered in the problem of vitamin contributions by the intestinal flora.

Experiments summarized in Table IV demonstrate roughly the sites of absorption of folic acid, biotin, and pantothenic acid in the intestinal tract. Comparing the amounts of these substances extractable by hot water found in the cecal walls (Table IV) with the amounts in the medium surrounding the bacteria (Table III, water extract) it is shown that the values are of similar magnitude, indicating a more or less free passage of the vitamins into the animal tissues. The results in both cases represent unbound vitamins calculated on the basis of moist weight of tissue. A further examination of Table IV reveals a considerably higher concentration of vitamin in the cecal wall than in the wall of the section just above it, the lower small intestine. Since bacteria are found in large quantities in the cecum and not further up the tract it is indicated that the unbound vitamins found in the cecal walls originated in the bacteria and not in the food. From a consideration of these experiments it appears evident that B vitamins are produced by rat intestinal flora and are absorbed into the tissues of the animals. The amount absorbed is a reflection of the amount of vitamin in the medium surrounding the bacteria and not of the total quantity synthesized by the bacteria.

The actual total quantity of each of the vitamins absorbed can be only approximated at best from these experimental results. This problem is complicated by the two unknown factors: possible synthesis of the vitamins by the body tissues and their destruction during metabolism. Exam-

ination of the $\frac{\text{output}}{\text{intake}}$ ratios in Table II demonstrates that pyridoxin, biotin, and, to a lesser extent, pantothenic acid, are excreted in excess of intake. On casual observation it might seem possible for rats with proper intestinal conditions and flora to obtain their entire requirement of these substances from intestinal bacteria. Such is often not the case since deficiencies in pyridoxin and pantothenic acid are readily obtainable. A reasonable explanation is that experimental diets used in producing the deficiencies may support a sparse, inadequate flora in the cecum.

The $\frac{\text{output}}{\text{intake}}$ ratios do not appear to have significance further than as a possible indication of the amount of destruction of the vitamins during metabolism. Such destruction has already been demonstrated by Leong (2) with thiamin and by Shourie and Swaminathan (6) on nicotinic acid.

Regardless of the specific significance of the $\frac{\text{output}}{\text{intake}}$ ratios, some approximate minimum values for bacterial contribution of vitamins can be calculated from these data and from information in Table III, if the possibility of tissue synthesis and metabolic destruction of pyridoxin can be neglected. On Diet A, Experiment II, it is shown that the output of pyridoxin is 11.5 γ per day with an intake of 3.5 γ per day. There must have been, therefore, a production of 8 γ per day by the intestinal bacteria. Referring to the value in Table III under "water extract," it appears that 0.96γ of pyridoxin is available from each gram of cecal contents. The 8 γ of pyridoxin therefore required a "turnover" of cecal contents corresponding to 8.3 grams per day. Using this value and the figures for available vitamins under Diet A, Experiment II, Table III, the following daily contribution of vitamins by intestinal bacteria can be calculated: inositol, less than 16 γ; nicotinic acid, 31 γ; pantothenic acid, 14 γ; riboflavin, 7.5 γ; thiamin, 6.6 γ; folic acid, 5.6 γ; pyridoxin, 8.0 γ; and biotin, 1.6 γ.

These values converted to percentages of each vitamin in the dietary intake, Diet A, Experiment II, are given in the first column, Table VI. A similar calculation of vitamins available to the rats, based on the pantothenic acid balance rather than the pyridoxin balance has been made (Diet B, Experiment I). These percentages are given in the second column of Table VI.

TABLE VI

Percentage of B Vitamins Supplied by Intestinal Flora

	Pyridoxin Basis	Pantothenic Acid Basis
Thiamin	18	8
Riboflavin	19	5
Nicotinic Acid	1.8	3.5
Pantothenic Acid	11	58
Pyridoxin	230	130
Biotin	230	420
Inositol	<0.08	<0.1
Folic Acid	67	71

A similar calculation using biotin as a basis gives lower values. None of the other vitamins shows a positive balance of $\frac{\text{output}}{\text{intake}}$ and thus cannot be used for such calculations. This is probably due to both metabolic destruction and a low output by the intestinal flora. In any event, the values given should represent minima since there is probably some metabolic destruction of pyridoxin and pantothenic acid.

The production of two types of flora was shown in Table V. In addition to this information it was found from plate counts that the cecum from rats on Diet A contained approximately 5×10^7 microorganisms, while the corresponding cecal contents from Diet B contained about 100×10^7 organisms. From Table III (water extract) it is observed that the vitamins available to the animals, per gram of cecal material, are about the same on both diets. On the other hand, from Table II, the $\frac{\text{output}}{\text{intake}}$ ratio is shown to be larger with Diet A than with Diet B. It therefore appears that the acidophylic flora is superior to the coliform flora due only to the much larger numbers of acidophylic organisms (20 times as many). Such a generalization is valid only for the conditions in question since minor variations in diet might change the picture radically.

Certain specific differences in vitamin contribution by bacteria, which can be unquestionably attributed to the type of flora, were found. From Table III (water extract) it can be observed that the acidophylic flora (Diet B) allows its pantothenic acid to diffuse much more freely into the surrounding medium than does the coliform flora (Diet A). A similar situation exists with folic acid, but to a lesser extent. Both of these results are confirmed by data in Table IV (cecum walls). An opposite effect is demonstrated with pyridoxin and thiamin where the vitamin availability is favored by the coliform type of organism. These results on rats probably cannot be carried over to other animals which are physiologically and anatomically different with respect to the support of intestinal flora.

SUMMARY

1. The absorption by the body tissues of B vitamins originating with intestinal bacteria in the rat cecum has been indicated.

2. Some approximate percentages of the dietary intake of B vitamins that are supplied by the intestinal flora are calculated: thiamin, 18%, 8%; riboflavin, 19%, 5%; nicotinic acid, 1.8%, 3.5%; pantothenic acid, 11%, 58%; pyridoxin, 230%, 130%; biotin, 230%, 430%; inositol, 0.08%, 0.10%; and folic acid, 67%, 71%. These are calculated on the basis of pyridoxin and pantothenic acid balances respectively.

3. The amount of vitamin contribution by the bacteria is markedly influenced by the type of flora which in turn may be modified by relatively small changes in diet. Both the type of flora and the number of organisms are important.

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THE VITAMIN REQUIREMENTS OF CECECTOMIZED RATS

By

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The results of recent investigations appear to have demonstrated that the intestinal flora of the rat synthesizes in varying degrees many of the B vitamins. Further, it has been shown that various diets may have a marked influence on the type and quantity of flora present in the cecum and colon and consequently on the production of the several vitamins concerned.

Mitchell and Isbell (1), (this bulletin, p. 125) have presented evidence which indicated that with the diets used substantial amounts of some of the B vitamins produced by bacterial synthesis were available to the rat. They showed that microorganisms in the cecum were mainly responsible for these additional sources of vitamins. Their investigations also emphasized the marked effect which diet has on both the quality and quantity of the intestinal flora.

It would seem that indication of vitamin synthesis by the intestinal flora of the rat could be directly substantiated by a study of the vitamin requirements of cecectomized rats. This type of experiment was carried out some years ago by Griffith (2). He reported only slight differences between the experimental and control rats in "B and G avitaminosis." However, the basal diet he used (purified casein 25 parts; sucrose 75 parts; salt mixture 4 parts; plus various supplements) may not have been favorable to the maintenance of bacterial growth in the cecum. Also, the vitamins riboflavin and thiamin, with which he was especially concerned, are among those which appear to be produced to a lesser degree in a usable form by the cecal flora (1).

Accordingly, we decided to check the value of the cecum as a vitamin source by testing the reactions of cecectomized and unoperated rats to a diet which would not inhibit the growth of the cecal bacteria and at the same time was deficient in the vitamins which seem to be produced in the cecum. Data were also obtained on the size of the cecum in relation to the size of the digestive tract in rats of varying ages.

EXPERIMENTAL

The weight of the cecum together with its contents and its relation to the weight of the digestive tract and contents was determined for rats of different ages and weights. A series of about 40 animals varying in size from 9.5 grams to 327 grams was used for this purpose. After ether anesthetization, the visceral cavity was opened up rapidly and the cecum ligatured off from the intestine. It was then removed and weighed. Next, the weight of the whole digestive tract from the esophagus to the anus was obtained. Extra fat and other accessory tissue were removed before weighing.

Experiment 1

Ten males, whose average weights were 100 grams, and ten females with average weights of about 90 grams, of the Wistar strain of albino rats were used in this study. These animals were 7 to 8 weeks old and together with the animals used in Experiment 2 represented a cross section of a number of litters born about the same time.

Two groups, consisting of 5 males and 5 females respectively, served as controls, and two similar groups were cecectomized and served as the experimental animals. Since the purpose of this experiment was to check the effect of cecectomy on rats maintained on an adequate diet, both control and experimental rats were placed on the regular Purina dog chow used for our stock animals and continued on this diet for a period of seven weeks.

Experiment 2

The animals used in this instance were litter mates of those utilized in Experiment 1. A group of 5 unoperated males and a group of five unoperated females were the controls. The experimental groups were made up in the same manner but using cecectomized rats.

Both controls and experimentals were placed on the pyridoxin-deficient ration II of Conger and Elvehjem (3). This diet consisted of sucrose 75 parts; casein 18 parts; corn oil 3 parts; plus supplements of thiamin, riboflavin, niacin, pantothen, choline, and liver extract. This experiment was continued for seven weeks.

Experiment 3

For this part of the investigation the two groups of unoperated and the two groups of cecectomized rats which had been on purina dog chow for seven weeks were used. At this time the males averaged about 238 grams and the females 163 grams in weight.

A "diluted" Purina diet was compounded for this experiment, which on the basis of the results obtained by Mitchell and Isbell, would give the unoperated rats very nearly their requirement of the B vitamins but which would leave the cecectomized animals deficient in several. Their results showed that with a diet of ground lean beef 75 parts; lactose 25 parts; or ground lean beef alone the daily contribution of the cecal flora appeared to be: inositol 16 γ; niacin 31 γ; pantothen 14 γ; riboflavin 7.5 γ; thiamin 6.6 γ; folic acid 5.6 γ; pyridoxin 8 γ; and biotin 1.6 γ. The dilute Purina diet was made up of powdered Purina dog chow 22 parts; sucrose 67 parts; casein 8 parts; salts 3 parts; and to each 100 grams of this was added riboflavin 400 γ; nicotinic acid 2,500 γ. Assuming that this diet enabled the cecum to produce quantities of vitamins comparable to the amounts found by Mitchell and Isbell; then these cecectomized rats had available in comparison to the unoperated animals: folic acid 26%, biotin 20%, pantothenic acid 69%, thiamin 55%, and pyridoxin 36%. This diet, it will be

noted, was somewhat deficient for the controls in pantothenic acid and thiamin if whole Purina is taken as the standard.

The animals were maintained on the diet for a period of 11 weeks, after which both control and experimental rats were sacrificed in order to obtain data on the internal organs and to check the state of the colon of the cecectomized groups.

Analyses were made of the content of some of the B vitamins in the feces of control and experimental groups during the course of the experiment.

RESULTS

Size of the cecum at various age levels

Table I and Figure 1 summarize the data obtained on the weight of the cecum and contents in relation to body weight and in relation to the digestive tract with its content in rats of different sizes. It will be noted that the cecum comprises only about 1% of the digestive tract in a 9.5 gram rat, while in a mature rat this organ is about one-third as large as the stomach, intestine and colon taken together. At the time of weaning the cecum is, in relation to the digestive tract, little more than one-fourth the size it will attain after the rat has passed the 100 gram size.

TABLE I

Cecum Weight in Relation to Body Weight and Digestive Tract Weight in the Rat

Number of Animals	Body Weight Grams	Digestive Tract Weight Grams	Cecum Weight Grams	Cecum to Body Weight Per Cent	Cecum to Digestive Tract Weight Per Cent
8	9.5	.53	0.0056	0.057	1.06
9	26.8	3.39	0.15	0.55	4.70
4	33.5	2.90	0.21	0.63	7.20
3	48.9	3.01	0.25	0.51	8.30
1	60.0	9.30	2.28	3.80	24.0
3	113.2	11.50	3.40	2.90	29.0
2	214.0	13.60	4.30	2.10	31.8
2	220.4	13.40	4.30	1.90	32.2
3	237.0	13.10	4.60	1.90	35.0
2	327.0	15.50	5.00	1.50	32.0

Cecectomized rats on Purina ration

Figure 2 shows the results of the experiment in which cecectomized and unoperated animals were maintained on the regular Purina ration. It will be seen that lack of a cecum did not handicap the rats on this diet during the seven weeks it was continued. The average gain per rat for the 5 males and 5 females in the operated group was 89 grams, while the gain in the similarly constituted control groups was 84 grams per animal. Careful examination of pelage, skin, feces, and blood hemoglobin disclosed no observable abnormalities in the cecectomized animals.

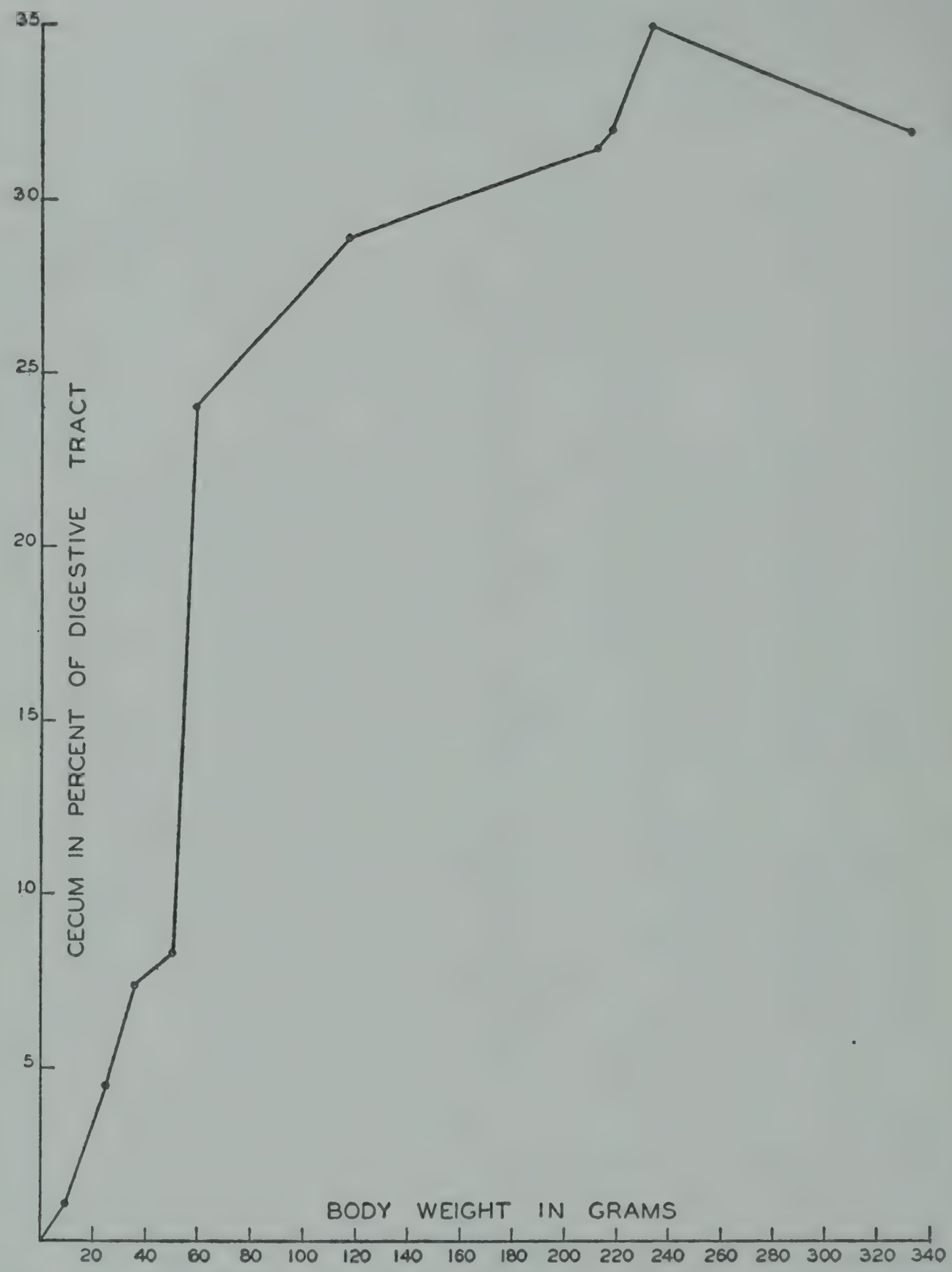

Fig. 1. Relation of Per Cent Weight of Cecum in Digestive Tract to Body Weight in Normal Rats.

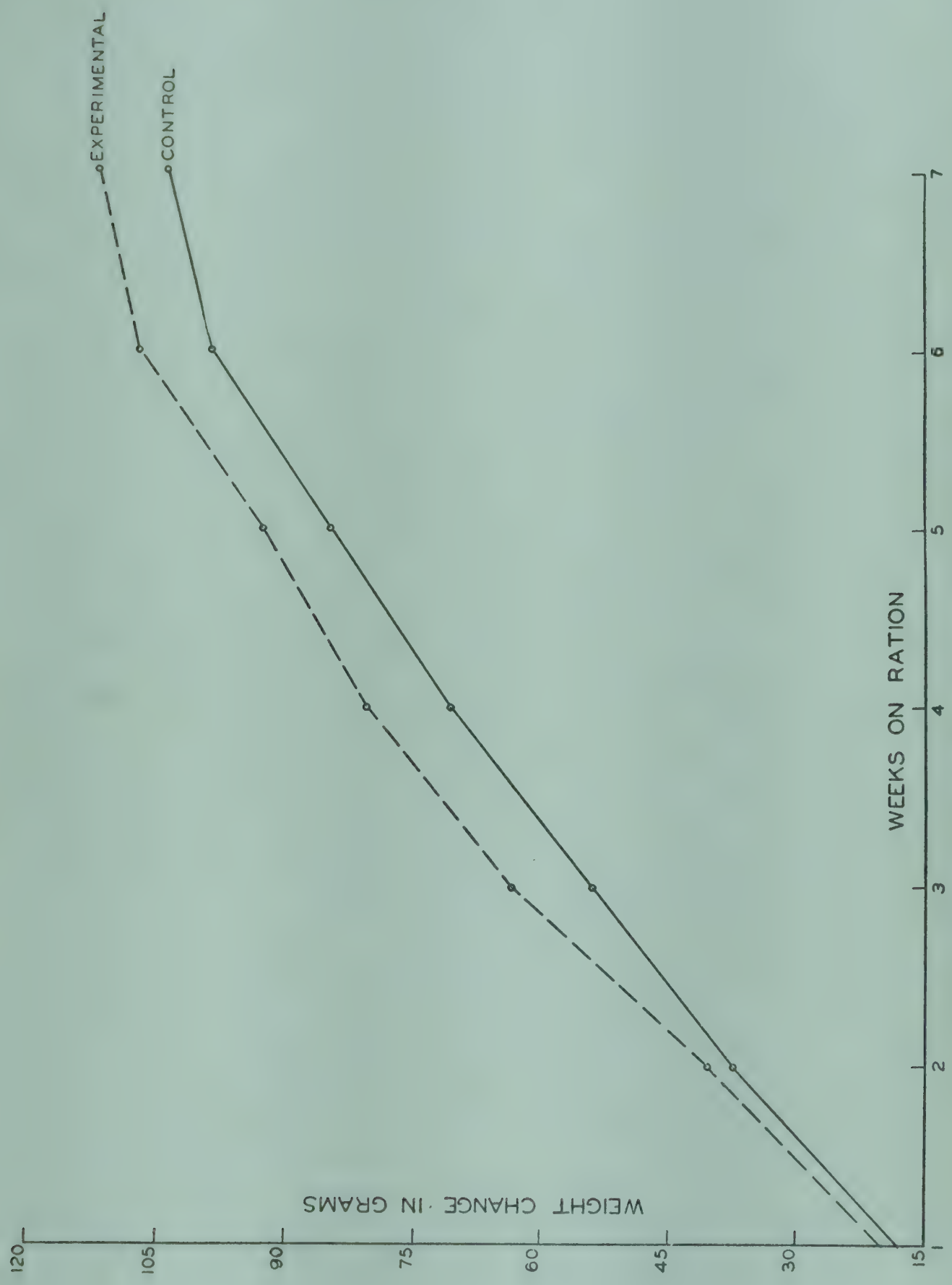

Fig. 2. Growth of Normal and Cecectomized Rats on Purina Ration.

Cecectomized rats on a B_6 deficient ration

Our results in this experiment were negative (Fig. 3). The growth rates of the operated and unoperated animals averaged about the same. This confirmed the results of Griffith's (2), whose basal diet was similar to the one used here.

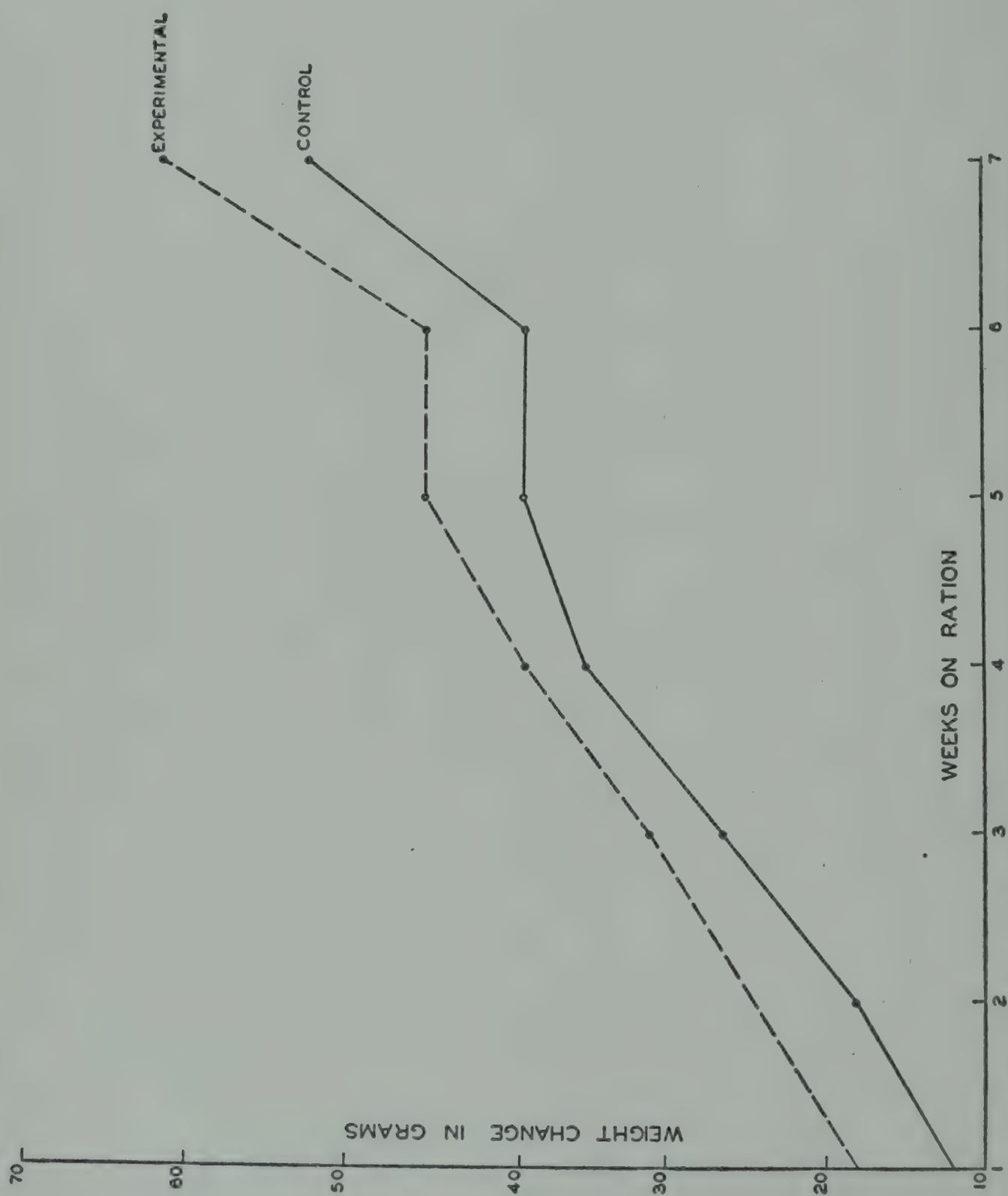

Fig. 3. Growth of Normal and Cecectomized Rats on Vitamin B_6 Deficient Ration.

Cecectomized rats on a "dilute" Purina ration

Rats lacking a cecum were severely handicapped by a "dilute" Purina diet compared to the unoperated animals. Figure 4 shows that after four weeks of this ration both cecectomized males and females began to lose weight. The males were much more severely affected in this regard than the females. During the 11 weeks this experiment was continued, the cecectomized males dropped from an initial average weight of 236 grams to 193 grams, an average loss of 43 grams. The control males initially weighed an average of 241 grams and gained 8 grams each to average 249 grams per rat at the conclusion of the experiment. The operated females lost an average of 1 gram per rat, going from 167 to 166 grams per animal.

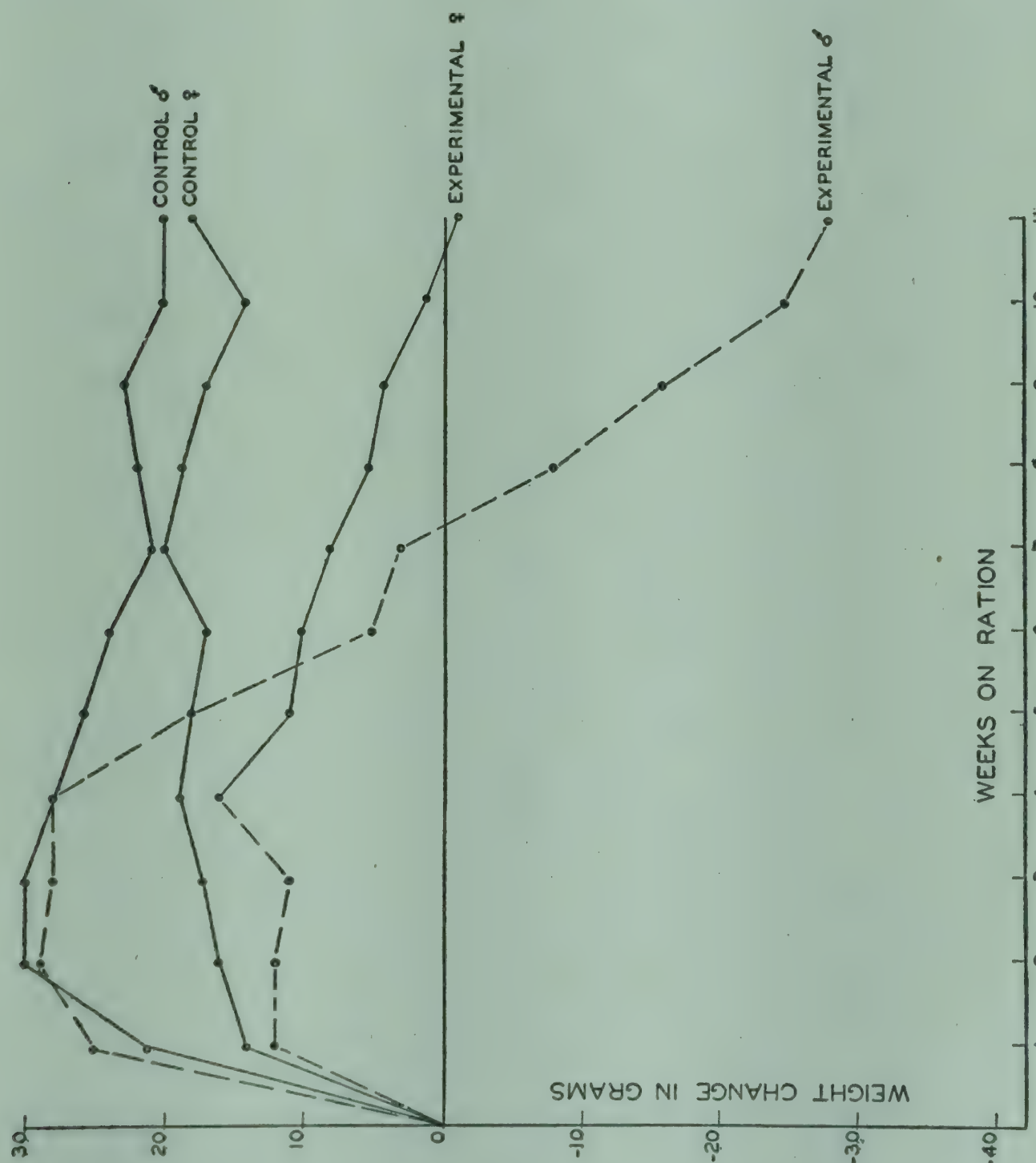

Fig. 4. Growth of Normal and Cecectomized Rats on "Dilute" Purina Ration.

The control females gained an average of 18 grams each, increasing from an average of 161 to 179 grams per rat. In Tables II and III the weight changes of each rat is recorded, together with data on the condition of the pelage and skin.

TABLE II

Individual Records of Control Male Rats on "Diluted" Purina Diet

	Initial Weight Grams	Weight at 11 Weeks Grams	Net Gain or Loss	Hemoglobin Grams Per Cent	Pelage	Epidermis
No. 1	260	286	+26	14.73	normal	normal
No. 2	226	235	+ 9	13.91	normal	normal
No. 3	255	271	+16	15.12	normal	normal
No. 4	239	215	—24	14.73	normal	normal
No. 5	226	236	+10	13.91	normal	normal
Average	241	249	+ 8	14.48		

Individual Records of Cecectomized Male Rats on "Diluted" Purina Diet

No. 1	214	184	—30	14.73	coarse; denuded areas; epidermosis	
No. 2	266	196	—70	14.92	coarse; denuded areas; epidermosis	
No. 3	248	176	—72	15.12	coarse; denuded areas; epidermosis	
No. 4	252	213	—39	14.30	coarse; sparse; epidermosis	
No. 5	200	196	— 4	13.72	coarse; denuded areas; epidermosis	
Average	236	193	—43	14.56		

TABLE III

Individual Records of Control Female Rats on "Diluted" Purina Diet

	Initial Weight Grams	Weight at 11 Weeks Grams	Net Gain or Loss Grams	Hemoglobin Grams Per Cent	Pelage	Epidermis
No. 1	149	155	+ 6	16.51	normal	normal
No. 2	164	186	+22	15.12	normal	normal
No. 3	164	183	+19	14.73	normal	normal
No. 4	185	200	+15	15.00	normal	normal
No. 5	144	170	+26	14.30	normal	normal
Average	161	179	+18	15.13		

Individual Records of Cecectomized Female Rats on "Diluted" Purina Diet

No. 1	177	195	+18	14.03	thin;	epidermosis
No. 2	172	170	— 2	15.19	thin	epidermosis
No. 3	170	162	— 8	14.50	denuded areas	epidermosis
No. 4	145	134	—11	13.91	thin	epidermosis
No. 5	171	169	— 2	14.81	thin	epidermosis
Average	167	166	— 1	14.49		

It will be noted that the control rats without exception appeared to have normal pelage and skin, while most of the cecectomized were partially denuded of hair, and all the experimental rats manifested an epidermosis which was characterized by the presence of brownish epidermal scales.

All of the animals in both control and experimental groups upon autopsy revealed normal feces, clear lungs and a hemoglobin level which was about the same for all groups. No visceral abnormalities were noted in any of these rats. It was observed that at the conclusion of the experiment the

cecectomized animals were listless and the males especially tended to be kyphotic. The controls appeared to be healthy, vigorous and unimpaired, even though the diet had not allowed them to make as much gain in weight as should have occurred during the period of the experiment. Examination of the colon and the juncture of the colon with the ileum in the cecectomized rats disclosed that some changes of a compensatory nature had occurred. The diameter of the upper colon in the operated rats averaged 0.58 cm. as compared to 0.40 cm. for the controls. Also, in some of the operated rats there was a tendency for an enlargement measuring up to 1 cm. in diameter to be situated at the point in the colon where the cecum had been joined to this organ. This compensatory hypertrophy was insignificant in comparison to the comparatively large space occupied by the cecum.

The results of feces' analysis are given in Table IV. These data show that the feces of the cecectomized rats contained in comparison to the

TABLE IV

Vitamins in the Feces of Cecectomized and Control Rats on Diluted Purina Ration

Vitamin	Control γ/gram	Cecectomized γ/gram	Cecectomized Where Control = 100	Estimated Cecectomized Where Control = 100
Folic Acid*	5.6	2.2	39	25
Pyridoxin	1.2	0.63	52	36
Pantothenic Acid	26.5	20.0	76	69
Thiamin	7.8	9.8	125	55

*Micrograms of material of "potency" 40,000.

controls: folic acid 39%, pyridoxin 52%, pantothen 76% and thiamin 125%. Thiamin, for some unknown reason, was higher in the feces of the cecectomized group.

DISCUSSION

The data indicate that the cecum does make a contribution to the rat's supply of some of the B vitamins, providing the diet is such as to support a cecal flora of the right quantity and/or quality. It is also evident that on a diet such as the pyridoxin-deficient ration II of Conger and Elvehjem (3), the cecum adds very little to the vitamin sources already contained in the diet. Finally, in this connection, it appears both from our data and those obtained by Griffith (2) that when a rat is maintained on an adequate diet, lack of a cecum is not a discernible handicap.

It is interesting to note that the cecum does not reach its maximum capacity until after the rat has passed the 100 gram size in weight. At the time of weaning, the rat's cecum is only about one-fourth as large relative to the digestive tract as it will be in the adult. This may account in part for the well recognized fact that rats at this stage are less resistant to some vitamin deficiencies than they are a week or so later.

Under natural conditions where the rat must compete for limited food supplies and where it is often necessary to subsist on a restricted diet for comparatively prolonged periods of time, it appears that the capacity of the cecum to contribute to the animal's vitamin supply would have distinct survival value. This is illustrated in the "dilute" Purina experiment in which the controls continued to appear normal in every way and yet the cecectomized animals on the same diet were severely affected. Under natural conditions these rats probably would not have survived for the duration of the experiment.

It is suggested that the cecectomized rat is a more suitable animal for vitamin investigations whenever the contribution of the cecum is in doubt. In studies concerned with the vitamin requirements of older animals, this type of rat would seem to be a necessity.

SUMMARY

A study was made of the relative size of the cecum in rats of different age levels and of the effect of cecectomy on the vitamin requirements of the rat.

The cecum and contents in per cent of the total digestive tract averaged 1% at the 9.5 gram level, 8% at the 59 gram level and more than 30% in the mature rat.

On an adequate diet, cecectomized rats did not appear to be handicapped when compared to unoperated animals.

Cecectomized and normal rats reacted similarly to the pyridoxin-deficient ration of Conger and Elvehjem. No cecal contribution to the deficient B vitamin was evident on this diet.

On a Purina ration diluted with sucrose and which was deficient in several of the B vitamins, the cecectomized rats were severely affected while the unoperated controls maintained good health.

It appears that where the diet supports the right intestinal flora, the cecum contributes to the rat's supply of several of the B vitamins.

It is suggested that the use of the cecectomized rat would lead to better controlled studies in B avitaminoses.

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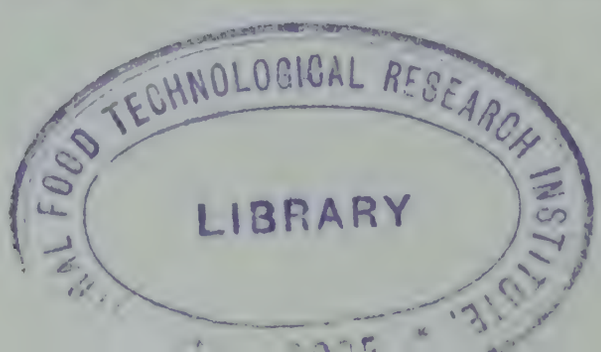

A SIMPLE MICROGRINDER FOR TISSUES

By

Herschel K. Mitchell

During the study of the vitamin content of rat and mouse tissues it became desirable to obtain equipment for grinding small amounts of tissues without loss. The grinder described was constructed for this purpose and has proven highly satisfactory. The apparatus is a modification of an ordinary meat grinder on a very small scale, the helical feeding device of the ordinary meat grinder being replaced by a metal plunger. The apparatus is shown in Figure 1. It is operated by placing the tissue sample in the barrel of the grinder, dropping the plunger on top, starting the motor and screwing down the cap until the plunger is in contact with the cutting blade. From a 200 mg. sample of mouse heart the grinder delivered 160 mg. of finely divided material. Most of the tissue remaining behind is retained between the cutting surfaces and could be reduced by using a thinner blade. A more complex and automatic apparatus involving a similar principle has been described by Seevers and Shideman (1). A simplified modification of this design has been constructed and used in this laboratory and has also been found to be highly satisfactory.

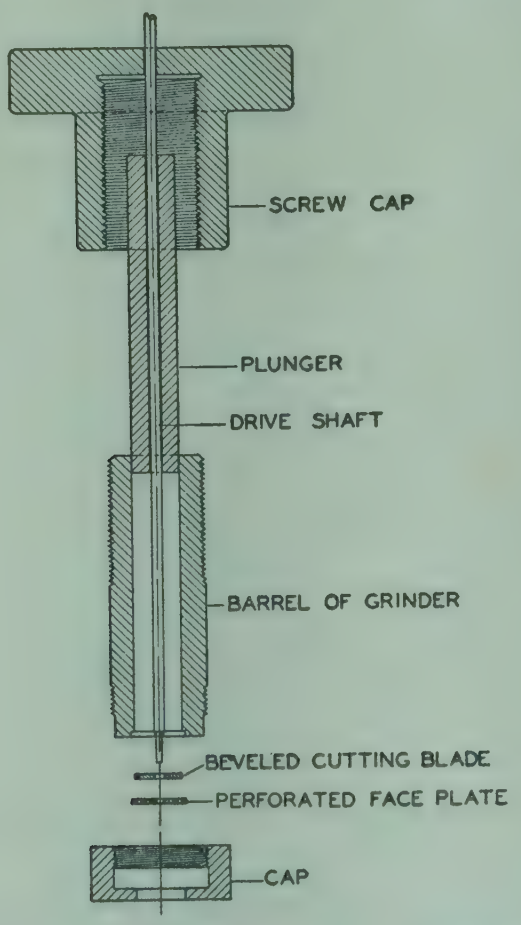

Fig. 1. A Microgrinder for Tissues.

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